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RELATIVE VARIABILITY OF INBRED LINES AND F_1 HYBRIDS IN LYCOPERSICUM ESCULENTUM

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Received May 20, 1960

AMONG outbreeding species of animals and plants, the nongenetic or environmental component of variability has been shown to be inversely proportional to the level of heterozygosity (MATHER 1950; ROBERTSON and REEVE 1952; LERNER 1953; MCLAREN and MICHIE 1956). Owing to fundamental dissimilarities in genetic architecture and behaviour between outbreeders and inbreeders, the relative variability of heterozygotes and homozygotes belonging to species with different breeding systems might be expected to show clear and constant differences. The information that is at present available on the relative variability of inbred and hybrid lines of inbreeding species does not, however, form a consistent pattern (LERNER 1953). This may be attributed partly to the fact that many of the data on variability have been presented in support of studies designed to answer other problems, with the result that it is not always possible to establish, with any reliability, the significance of the published estimates. When this is recognized, and only the most reliable data from the standpoint of experimental layout are admitted, a somewhat less contradictory picture emerges.

The evidence from studies on tomato (POWERS 1942), on Galeopsis (HAGBERG 1952), and on Nicotiana species (JINKS and MATHER 1955; PAXMAN 1956; SMITH and DALY 1959), all show that the variability of F_1 hybrids in the inbreeding species studied fall within the range of the inbred parents. The most serious challenge to this conclusion is found in the extensive study of mutants in barley (ROBERTSON and AUSTIN 1935) and in their further analysis and interpretation by GUSTAFSSON (1946). The ratio of the coefficient of variation between homozygotes and heterozygotes in the barley material suggests that the heterozygotes were more variable, but the average ratio over all comparisons was very close to unity, 1.067 ± 0.025. It is doubtful whether a difference in variability of this magnitude has any biological meaning even if it is proved to be real. LEWIS (1954), following a study of flower number in the tomato and an examination of other published data, attempted to generalize the behaviour of inbreeding species by correlating the level of variability directly with dominance. Since only the one character which LEWIS himself studied showed dominance of the smaller parent, most of the relative variabilities on which his conclusions were based might simply be a reflection of a positive relation between the variance and the mean.

The present study on eight inbred lines and six hybrids in the tomato was undertaken as a further contribution to the understanding of patterns of varia-

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bility in inbreeders, and the design chosen for the experiments was such as would provide the replication necessary to make valid comparisons between the calculated estimates of variability.

MATERIAL AND METHODS

The material comprised eight inbred lines intercrossed to give the six F_1 hybrids, $XT/_1$, $XT/_2$, $XT/_3$, $XT/_{19}$, $XT/_{23}$ and $XT/_{27}$, as shown in Tables 1 and 2 of the text. The inbred lines represent commercial varieties which have been multiplied by selfing over very many generations, and their breeding behaviour (which is typical of an obligate, inbreeding species) indicates that they are pure lines. Two trials, each consisting of four inbreds and three hybrids, were conducted. The first (experiment 1) was sown on the 20th April, 1959, to take advantage of the high light conditions of early and midsummer, and the second (experiment 2) was commenced on the 10th June, 1959, to provide a contrast in growing conditions. All the material was grown under glass where variability,

TABLE 1a

Means and their standard deviations of the ten replicate means of four inbred lines and three F_1 hybrids in experiment 1

	59	×	42	×	5	×	18
Character	—	$XT/_1$	—	$XT/_2$	—	$XT/_{27}$	—
No. of fruit	11.74±0.55	20.93±0.96	22.41±1.01	20.76±1.36	13.83±0.79	16.22±0.88	11.30±0.79
Av. wt. per fruit	4.14±0.011	2.52±0.07	2.44±0.07	2.61±0.05	3.54±0.09	3.54±0.08	3.27±0.08
Wt. per plant	47.80±3.28	49.66±3.40	50.79±4.77	49.98±4.04	44.96±3.38	50.21±4.01	31.07±2.67
Flower no.	7.67±0.18	9.19±0.38	9.19±0.28	9.55±0.24	9.31±0.30	9.15±0.29	9.87±0.22
Flowering date	3.80±0.19	2.88±0.15	6.99±0.27	3.90±0.23	3.43±0.20	3.74±0.18	4.71±0.18

TABLE 1b

Relative variability of different characters of parents and F_1 hybrids in experiment 1 summarized from Table 1a

Character	Mean σ^* of least variable parent	Mean σ^* of most variable parent	Observed mean σ^* of F_1 hybrids
No. of fruit	0.71	0.94	1.07
Av. wt. per fruit	0.08	0.10	0.07
Wt. per plant	3.11	4.31	3.82
Flower no.	0.23	0.30	0.31
Flowering date	0.19	0.25	0.19
All characters	0.86	1.18	1.09

* $=\sigma$ of mean of ten replicate means.

TABLE 2a

Means and their standard deviations of the seven replicate means of four inbred lines and three F_1 hybrids in experiment 2

Character	42 —	× XT/$_3$	125 —	× XT/$_{23}$	7a —	× XT/$_{19}$	59c —
No. of fruit	14.95±0.63	14.59±0.54	8.51±0.37	11.63±0.49	9.06±0.72	11.17±0.61	8.01±0.34
Av. wt. per fruit	1.54±0.06	1.86±0.04	2.59±0.42	2.37±0.08	2.02±0.08	2.39±0.09	3.17±0.13
Wt. per plant	22.98±1.52	26.83±1.27	21.23±1.68	27.33±2.13	18.32±2.31	26.84±2.30	25.06±1.70

TABLE 2b

Relative variability of different characters for parents and F_1 hybrids in experiment 2 summarized from Table 2a

Character	Mean σ* of least variable parent	Mean σ* of most variable parent	Observed mean σ* of F_1 hybrids
No. of fruit	0.36	0.69	0.55
Av. wt. per fruit	0.08	0.32	0.07
Wt. per plant	1.63	2.10	1.90
All characters	0.69	1.04	0.84

* $=\sigma$ of mean of seven replicate means.

due to environmental fluctuations, is high as compared with open ground. Experiment 1 consisted of ten replicates of 20 plants in each plot set out in randomized complete blocks in various parts of the glasshouse area. The position of the blocks represented quite wide differences in site aspect and type of glasshouse and, therefore, in the light and temperature conditions under which they were grown. Experiment 2 differed from above in that only seven replicates were available and the number of plants per plot varied between 20 and 36.

Five characters were recorded on each plant in experiment 1 and three in experiment 2. The data on fruit characters refer to records over the first three weeks of fruiting, and all weights are given in ounces. Flower number refers to the number of flowers on the first inflorescence, and the figure for flowering date is the mean for the first flower on the first florescence. Plants that flowered on the first day were scored 1, those on the second day were scored 2, etc., until flowering was complete.

RESULTS

Variability of plot means: The stability of plot (population) means, over a range of different conditions, reflects the ability of the genotype to direct and maintain the development of a large number of individuals within certain limits. Given a character with a symmetrical curve of distribution, uniformity among

means of genetically identical populations would indicate that development is regulated by the genotype along one predominant pathway and at one predominant rate, irrespective of environmental influences. Where the character distribution is skewed, the use of means to compare interpopulation stability is less informative, and the mode would be more meaningful statistically in respect of developmental processes. But since previous work on this subject has been based on the assumption that the frequency distributions of quantitative characters are usually normal and discussion has centered exclusively on comparison of means, the use of the mode in the treatment of the present data was not adopted.

A measure of the relative stability of the inbred lines and the hybrids, over the different conditions under which the replicates were grown, is given by the standard deviation of the mean of ten plot means of each line in experiment 1 and of seven plot means in experiment 2. These are presented in Tables 1 and 2.

Of the 24 comparisons of stability between F_1 hybrids and their parents in Tables 1a and 2a, 15 of the hybrids fall within the range of the parents in respect of the variability of the mean of the replicates. The estimates for the remaining nine hybrids fall on either side of the parental range—four being above and five below the range of their parents. None of these, however, differed greatly from the corresponding highest or lowest parent. The relative stabilities for the different characters in the two experiments can be assessed from Tables 1b and 2b. Apart from fruit number in experiment 1 the mean standard deviation of characters in F_1 hybrids do not fall outside the values of the parents, and the average for all characters is very close to the midparental point. There is no suggestion in this material that hybrids are any more, or any less stable over the range of environments than are the inbred parents. It is worth noting that in respect of the characters, average weight per fruit and flowering date, the hybrids are as stable as the most stable parent, whereas other characters are either intermediate in this respect, or else show "dominance" in the opposite direction. It seems likely that the direction and degree of "dominance" in respect of stability differs for different characters, and therefore, that the degree of stability of the F_1 hybrid is a specific property established by the parental genotype rather than that of a genotypic state such as heterozygosis.

Variability of individuals in one environment: Comparisons of the standard deviations of individual plot means (Tables 3, 4 and 5) gives a measure of the relative stability of the different genotypes in the face of small, random, fluctuations during development. Whereas the estimates of variability of the different means examined in the previous section is an attempt to measure the rigidity of genotypic regulation of the mean developmental pathway in various populations, the variability of individual means measures the latitude allowed by a genotype to each individual in development.

Of the 11 F_1 means which fall outside the range of the parents in the 24 comparisons given in Tables 3 and 4 only three (fruit number in $XT/_{27}$ and flower number in $XT/_1$ and $XT/_2$) are significant using the F test at one percent probability. It will be shown in Table 6 that differences in levels of variability in

TABLE 3

Mean standard deviations and their standard deviation of the ten replicates of four inbred lines and three F_1 hybrids in experiment 1

Character	59	×	42	×	5	×	18
	—	$XT/_1$	—	$XT/_2$	—	$XT/_{21}$	—
No. of fruit	2.11±0.25	3.04±0.15	3.97±0.37	3.37±0.33	2.71±0.26	3.49±0.22	2.83±0.21
Av. wt. per fruit	0.39±0.04	0.46±0.01	0.28±0.01	0.24±0.02	0.26±0.04	0.40±0.03	0.48±0.03
Wt. per plant	7.63±0.83	7.08±0.67	7.68±0.88	8.54±1.27	9.24±0.96	9.46±0.91	7.16±0.58
Flower no.	1.81±0.16	2.61±0.21	1.79±0.12	2.40±0.20	2.02±0.22	2.24±0.24	3.05±0.19
Flowering date	0.66±0.06	0.70±0.04	2.16±0.21	1.05±0.05	0.90±0.13	0.82±0.07	1.13±0.09

TABLE 4

Mean standard deviations and their standard deviation of the seven replicates of four inbred lines and three F_1 hybrids in experiment 2

Character	42	×	125	×	7a	×	59c
	—	$XT/_2$	—	$XT/_{23}$	—	$XT/_{19}$	—
No. of fruit	3.33±0.31	3.25±0.29	2.68±0.05	2.81±0.23	2.37±0.25	2.32±0.34	1.83±0.04
Av. wt. per fruit	0.21±0.02	0.30±0.02	0.53±0.04	0.43±0.02	0.32±0.02	0.32±0.04	0.55±0.04
Wt. per plant	4.95±0.44	5.59±0.49	5.64±0.32	6.33±0.76	5.07±0.79	5.46±0.59	5.21±0.55

TABLE 5

Relative variability of different characters for parents and F_1 hybrids summarized from Tables 3 and 4

Character	Mean σ^* of least variable parent	Mean σ^* of most variable parent	Observed mean σ^* of F_1 hybrids
No. of fruit	2.40	3.19	3.05
Av. wt. per fruit	0.28	0.46	0.36
Wt. per plant	6.26	7.11	7.08
Flower no.	1.87	2.29	2.42
Flowering date	0.82	1.82	0.86
All characters	2.57	3.20	3.03

* $=\sigma$ of single plot means.

TABLE 6

Summary of covariance, analysis of means and standard deviations in experiments 1 and 2

Character	Experiment no.	Coefficient of correlation	Coefficient of regression	Variance ratio (for testing adjusted means)
Fruit no.	1	0.1687	0.0438±0.033	...
	2	0.0717	0.0265±0.057	...
Av. fruit weight	1	−0.0819	−0.0291±0.045	...
	2	0.4704	0.1684±0.049	3.19
Wt. per plant	1	0.2411	0.0592±0.030	...
	2	0.4441	0.1377±0.043	0.64
Flower no.	1	0.3715	0.1380±0.044	1.14
Flowering date	1	0.6553	0.4471±0.063	5.36

For r, $n = 68$ in experiment 1 and 47 in experiment 2.
For V.R. $n_1 = 6$ and n_2 62 in experiment 1 and $n_1 = 6$ and $n_2 = 41$ in experiment 2.

respect of flower number are entirely dependent on the magnitude of the means. There is, therefore, no evidence to suggest that the heterozygous individuals possess any characteristic stability over and above that which they inherit from both parents. It will be noted from Table 5 that the stability of flowering date among individuals shows a decided "dominance" of the least variable parent. The same trend in the direction of "dominance" was observed in respect of the stability of plot means for this character (Table 1b). Most other characters showed "dominance" in the opposite direction, which also agrees with the observations on the different plot means.

Variability and the magnitude of the mean: The extent to which variability is dependent on the size of the mean was tested by covariance analysis. A summary is presented in Table 6.

From the data given in the previous tables there appeared to be no reason to suspect a difference between the inbred lines and the hybrids in respect of possible correlations between the means and their standard deviations. The data from all the lines were, therefore, analyzed together for covariance. Four clear instances of correlation between the mean and the standard deviation are established by the analysis. In two of these, weight per plant in experiment 2 and flower number in experiment 1, the variance ratio for testing significance of adjusted plot means shows that the difference between the standard deviations of the different lines can be attributed entirely to differences in their means. The variance ratios for the other two characters which showed a correlation between the mean and the standard deviation suggest that the latter differ to a greater extent than would be expected if the differences were entirely due to the level of the means. In other words, the standard deviation of the different stocks are inherently different, as well as being tied to the magnitude of character expression.

Where there is no correlation between the means and the standard deviations, relative variability can be determined by direct comparisons of the standard deviations given in Tables 3 and 4. Of the characters which behave in this way, only fruit number in the hybrid $XT/_{27}$ has a significantly different level of

variability when compared with both parents. Differences in variability between inbred lines are significant in many cases.

DISCUSSION

The present data together with those quoted previously for other inbreeding species emphasize the difference that exists between inbreeders and outbreeders in respect of the control of enviromentally induced variability. The mechanism governing the various forms of stability or flexibility which have been recognized in treatments of this subject (see THODAY 1953) are so imprecisely understood, and the evolutionary meaning of variability estimates is so vague (see SIMPSON 1944, pp. 37–42) that a general discussion on the nature and significance of this difference would not seem justified. Nevertheless, there are a few points that appear noteworthy. In discussing the greater developmental homeostasis of heterozygotes in outbreeders, LERNER (1953, p. 108) stressed that the condition was a manifestation of previous selection, and that departure from the breeding system that is normal for the species leads to reduced buffering of developmental process in individuals and, therefore, to greater variability resulting from differences in the environment. On the basis of this hypothesis one is led to expect that in inbreeders, where selection operates mainly on homozygotes, and where hybrids represent a complete departure from the normal breeding system, most inbred lines would show less variability than the F_1 hybrid both on the intra- and interpopulation level. Clearly, either the argument developed to explain the variability characteristics of heterozygotes in outbreeders is false, or else the differences in genetic structure resulting from the two breeding systems lead, under selection, to a different pattern of variability control. Of these two possibilities the latter appears to provide the more likely explanation for the failure of extrapolation from the behaviour of outbreeding species. One obvious, relevant difference between the two breeding systems is that homozygotes in outbreeders are relatively more frequent than heterozygous combinations in inbreeders. In the former, where fitness depends on a certain level of obligate heterozygosity, homozygous combinations will appear relatively frequently through segregation, and, thus, selection will operate on both genotypic classes. In obligate inbreeding species, on the other hand, heterozygotes appear only rarely, being dependent only on the rate of mutation. Furthermore, the chance of survival of the rare heterozygote in an inbreeder is extremely low; it will be rapidly eliminated following self-fertilization. Thus in species where self-fertilization is obligatory, selection will operate almost exclusively on homozygous genotypes. The almost complete absence of the heterozygote in inbreeders may explain why the consequences of homozygosity and heterozygosity in respect of the control of variability is not as sharply defined in inbreeders as it is in outbreeders. It may also provide a clue to the cause of the apparent contradiction between breeding systems in relation to LERNER's general thesis on developmental homeostasis in cross-fertilizing species.

A brief reference may be made to the observations made in the present material on the control by the genotype of the environmental, or nongenetic com-

ponent of variability. Similar observations have been reported by GILBERT (1960). The inbred, homozygous lines used in the present experiments showed significant differences in interplant variability, and for flowering date there was evidence that the variability level of the least variable parent was transmitted as a "dominant" character. Differences in variability in this material must therefore be due to differences in gene content and not to states of homozygosity or heterozygosity of the genotype. Variability, like everything else, is under the control of the gene, and in inbreeding species no great advantage in exercising the control belongs to either of the genotypic states. Thus, hybrid lines of naturally self-fertilizing species, such as the tomato, do not possess intrinsic advantages in uniformity of behaviour such as have contributed to the success of F_1 hybrids in outbreeding species of crop plants.

SUMMARY

(1) The relative level of nongenetic variability in respect of five quantitative characters has been compared in six F_1 hybrids and eight inbred lines of the tomato.

(2) Both the inter- and intrapopulation level of variability of the F_1 hybrids fell within the range of their respective parents. None of the data suggests any intrinsic difference between inbred lines and hybrids in respect of their ability to buffer or to eliminate the variability that is induced by the environment.

(3) In four out of eight instances, the level of variability for certain characters was shown to be correlated with the magnitude of the mean. In two cases where there was significant correlation between the mean and the standard deviation, the differences in variability could not be entirely explained by differences in the means, and in these, different homozygous genotypes conditioned different levels of variability irrespective of the size of the mean.

(4) The characteristic levels of variability of certain parents were found to be transmitted to the F_1 hybrids. Generally, the variability of F_1 hybrids fluctuated around the mid parental value, but in respect of flowering date low variability was transmitted as a dominant character.

(5) These findings are briefly discussed in relation to the known variability patterns of outbreeding species, and possible reasons for differences in variability patterns resulting from the two breeding systems are suggested.

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INTERFERENCE PATTERNS IN FAMILY Y-1 OF SACCHAROMYCES[1]

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THE chromosome maps of Saccharomyces in the Lindegren Breeding Stock show the locations of 20 genes (LINDEGREN, LINDEGREN, SHULT and DESBOROUGH 1959). The present communication is a detailed analysis of one family (Y-1) heterozygous for ten genes not yet located in the chromosome maps, as well as five established genes. The genetic factors controlling resistance and sensitivity to actidione, 3-amino-1, 2, 4-triazole and allyl glycine are of special interest Sensitivity or resistance to actidione has been shown to be under the control of two genes located on different chromosomes (LEE 1959). Only one gene (*AC-1*) is studied in this family since genotypes within tetrads from a hybrid heterozygous for both genes are not distinguishable. That the phenotypes of cultures resistant or sensitive to allyl glycine and 3-amino-1, 2, 4-triazole are each controlled by a single gene is shown through tetrad analysis in this family. The gene *AD-a* controlling the synthesis of adenine is not associated with any visible coloration and is recorded in this breeding stock for the first time. (The adenine locus corresponding to this mutant has not yet been determined (ROMAN 1956).) Position of genes not previously located on the chromosome maps is attempted through tetrad analysis.

MATERIALS AND METHODS

Two haploid cultures, 24383 and 26466, were selected as parents from the Lindegren Breeding Stock. These two cultures have contrasting genotypes for 15 different genes as follows:

24383: *a su GA MG AD-a UR is ar ly ph NA ag ni ac-1 tz*
26466: *α SU ga mg ad-a ur IS AR LY PH na AG NI AC-1 TZ*

Table 1 gives the phenotypes corresponding to the above genotypes.

Cultures 24383 and 26466, grown on two percent glucose-nutrient agar for 24 hours at 30°C, were both inoculated into liquid nutrient medium. When there was 85 percent fusion between the cells of the two parental cultures, they were harvested and transplanted onto sporulation agar medium (MCCLARY, NULTY and MILLER 1959). The cultures were then incubated at 27°C for 48 hours after which time ascospores were readily detectable.

Only four-spored asci were selected for dissection with a micromanipulator. Isolated ascospores from 140 asci were then transplanted to nutrient agar. Of

[1] This work has been supported by a research grant from the U.S. Public Health Service C-4682.

[2] Rockefeller fellow, Hokkaido University, Sapporo, Japan.

TABLE 1

Genotype symbols with their corresponding phenotypes

Genotype	Phenotype
α/a	mating types
SU/su	fermentation/nonfermentation of sucrose
GA/ga	fermentation/nonfermentation of galactose
MG/mg	fermentation/nonfermentation of methyl-α-D-glucopyranoside
AD-a/ad-a	nonrequirement/requirement of adenine
UR/ur	nonrequirement/requirement of uracil
IS/is	nonrequirement/requirement of isoleucine
AR/ar	nonrequirement/requirement of arginine
LY/ly	nonrequirement/requirement of lysine
PH/ph	nonrequirement/requirement of phenylalanine
NA/na	nonrequirement/requirement of nicotinic acid
AC-1/ac-1	resistance/sensitivity to 10–40 ppm actidione
AG/ag	resistance/sensitivity to 200–500 ppm allyl-glycine
NI/ni	resistance/sensitivity to 1–10% nicotine
TZ/tz	resistance/sensitivity to 1.5% 3-amino-1, 2, 4-triazole.

these, all four spores survived in 130 tetrads, while only three spores survived in each of the remaining ten tetrads.

Modified Burkholder's medium (MIDDLEKAUFF, HINO, YANG, LINDEGREN and LINDEGREN 1957) was employed to determine the culture's requirement of specific nutrients. In this cross, cultures were tested for requirement or non-requirement of seven different nutrilites: adenine, uracil, isoleucine, arginine, lysine, phenylalanine and nicotinic acid. Tests for fermentation of sucrose, galactose and α-methyl-D-glucopyranoside were performed by the Durham tube technique (LINDEGREN 1956). Mating type was determined by the standard method (LINDEGREN 1949a).

Resistance and sensitivity to nicotine and actidione were tested by methods described in LINDEGREN, LINDEGREN and DESBOROUGH (1959) and MIDDLEKAUFF *et al.* (1957) and have been demonstrated within the Lindegren Breeding stock to be gene controlled. In addition to these, resistance and sensitivity to allyl glycine (SARACHEK and FOWLER 1959) and 3-amino-1, 2, 4-triazole revealed 2:2 segregation in the tetrads of this family. Here, the test tube method described in MIDDLEKAUFF *et al.* (1957) was employed using 200–500 ppm of allyl glycine and 1.5 percent 3-amino-1, 2, 4-triazole as additives to the modified Burkholder's Medium.

Genetic analysis of Y-1

Each ascospore culture from the 140 asci dissected in family Y-1 was tested for the 15 gene markers: *α*, *SU*, *GA*, *MG*, *AD-a*, *UR*, *IS*, *AR*, *LY*, *PH*, *NA*, *AG*, *NI*, *AC-1* and *TZ*. The tetrads were then classified for all possible combinations of two genes. For two genes there are three types of unordered tetrads: parental ditype (I), nonparental ditype (II), and tetratype (III). For example, the hybrid for this family is *a su* × *α SU*, and for this specific pair of genes the type I, II and

III tetrads are: *a su, a su, a SU, a SU; a SU, a SU, a su, a su;* and *a su, a SU, a SU, a su*, respectively. When the number of tetrads of each type has been counted, the resulting ratio of I:II:III is called the tetrad distribution. There are $15 \times 14/2 = 105$ tetrad distributions for this family; these are given in Table 2.

The relative frequency of type I, II and III tetrads are denoted a_1, a_2, and a_3 (where $a_1 + a_2 + a_3 = 1$), respectively. If two genes segregate independently, the ratio I:II:III will be 1:1:4, i.e., $a_1 = a_2$, $a_3 = 2/3$. A tetrad distribution which does not deviate significantly from this ratio is designated an N distribution. Genes linked on the same chromosome usually produce a significant deviation from this distribution, characterized by $a_1 > a_2$ and $a_3 < 2/3$. Such a distribution is designated an L distribution. There are other ways in which a tetrad distribution may deviate from the 1:1:4 ratio. If the number of type I tetrads does not deviate significantly from the number of type II tetrads, but the number of type III tetrads is significantly less than two thirds of all tetrads ($a_1 = a_2$, $a_3 < 2/3$), the distribution is called an F distribution. Such a distribution is typical of genes located near the respective centromeres of two non-homologous chromosomes which segregate independently at meiosis I. If it is assumed that chromosomal and chromatid intereference are absent, the "distance" (that is, 50 × the average number of crossovers per meiosis) is given by $D_1 = -50\ 1n\ (a_1 - a_2)$ (Trow 1913; Haldane 1919) or $D_2 = -33.3\ 1n\ (1 - 3a_3/2)$ (Rizet and Engelmann 1949). This unit has been designated "strane" (Lindegren *et al.* 1959) after Sturtevant, Trow, and Haldane. The factor 50 makes the unit directly comparable with centimorgans. For regions $\leqslant 15$ stranes, stranes $\approx$ centimorgans. The first formula (D_1) is applicable to L distributions while the second formula is applicable to *both* L and F distributions. (In the latter case, D_2 measures the average number of exchanges per meiosis occurring in the two centromere proximal regions of each chromosome (Whitehouse 1957). The advantage of using D_1 and D_2 units, instead of centimorgans lies in the fact that nonadditivity of these units is *direct evidence of interference*. Furthermore, for a given region, disagreement between D_1 and D_2 is evidence of intraregional interference. In fact, the equation $D_1 = D_2$ is equivalent to Papazian's formula (1951).

If the frequency of tetratype tetrads significantly exceeds 2/3, the distribution is designated a T distribution. (For examples of these distributions see Rizet and Engelmann 1949; Sager 1955.) If it is also true that $a_1 > a_2$, the distribution is denoted LT.

Because of the possibility of both chromosomal and chromatid interference as well as preferential segregation of centromeres, a given type of tetrad distribution may have more than one explanation. For example, it is possible that an F distribution in which $1/2 < a_3 < 2/3$ may result for genes on the same chromosome when there is an excess of 4-strand doubles, a deficiency of 3-strand doubles, or even negative chromosomal interference. (The data of Ebersold (1956) illustrates this situation.) Conversely, an L distribution may result for genes on *different* chromosomes if the centromeres of these chromosomes segregate preferentially in parental combination. Further tests may be used to distinguish these possibilities. Thus, in the former case 3-point tetrad data is helpful in elucidating

TABLE 2

Tetrad distributions from family Y-1

Gene pair	I	II	III	Gene pair	I	II	III
a SU	21	29	87	*AD-a NA*	16	22	90
a GA	54	42	47	*AD-a AG*	22	18	87
a MG	19	26	88	*AD-a AC-1*	19	18	84
a AD-a	28	20	86	*AD-a NI*	16	30	81
a UR	49	42	47	*AD-a TZ*	11	14	43
a IS	23	26	91				
a AR	27	27	91	*UR IS*	24	10	96
a LY	22	24	94	*UR AR*	29	21	87
a PH	28	35	77	*UR LY*	22	26	89
a NA	30	18	88	*UR PH*	22	34	77
a AG	20	25	86	*UR NA*	29	22	77
a AC-1	41	41	61	*UR AG*	21	8	98
a NI	19	32	86	*UR AC-1*	28	40	59
a TZ	9	12	53	*UR NI*	21	20	90
				UR TZ	12	0	60
SU GA	28	25	82				
SU MG	25	32	82	*IS AR*	22	34	80
SU AD-a	20	34	80	*IS LY*	17	32	96
SU UR	28	19	86	*IS PH*	19	27	88
SU IS	28	20	85	*IS NA*	25	25	80
SU AR	24	25	91	*IS AG*	122	1	12
SU LY	34	10	93	*IS AC-1*	19	16	84
SU PH	16	20	89	*IS NI*	97	1	23
SU NA	16	22	97	*IS TZ*	55	0	15
SU AG	18	24	87				
SU AC-1	27	29	69	*AR LY*	17	33	92
SU NI	24	21	89	*AR PH*	24	14	93
SU TZ	12	11	51	*AR NA*	28	26	80
				AR AG	23	31	74
GA MG	20	28	94	*AR AC-1*	18	21	91
GA AD-a	23	21	89	*AR NI*	23	28	84
GA UR	50	57	34	*AR TZ*	15	14	45
GA IS	20	18	98				
GA AR	34	20	88	*LY PH*	25	20	91
GA LY	27	22	90	*LY NA*	18	26	90
GA PH	26	23	89	*LY AG*	21	21	87
GA NA	28	29	81	*LY NI*	17	26	90
GA AG	19	15	95	*LY AC-1*	29	14	85
GA AC-1	74	4	50	*LY TZ*	11	16	47
GA NI	19	21	95				
GA TZ	7	8	61	*PH NA*	27	18	57
				PH AG	16	24	94
MG AD-a	20	20	92	*PH AC-1*	19	26	80
MG UR	24	20	90	*PH NI*	25	28	78
MG IS	20	20	97	*PH TZ*	14	12	45
MG AR	23	28	88				
MG LY	31	16	90	*NA AG*	24	22	81
MG PH	30	21	85	*NA AC-1*	16	24	81

MG AG	19	16	98	*NA NI*	18	22	91
MG NA	16	28	88	*NA TZ*	11	10	48
MG AC-1	20	29	81				
MG NI	22	20	92	*AG AC-1*	20	14	86
MG TZ	12	12	50	*AG NI*	96	0	31
				AG TZ	50	0	14
AD-α UR	28	21	79				
AD-α IS	25	20	86	*AC-1 NI*	22	17	86
AD-α AR	27	14	90	*AC-1 TZ*	9	10	48
AD-α LY	17	26	80				
AD-α PH	19	21	91	*NI TZ*	40	1	27

the interference pattern. In the latter case an additional gene, linked to an opposite arm of one of the centromeres, will yield significantly *nonadditive* distances (the total distance, however chosen, being too short) for both D_1 and D_2, giving the artificial appearance of a "branched" linkage map when these distances are taken literally.

Two-point analysis

Centromere linkage: Typical F distributions were obtained for the three centromere markers α, *GA*, and *UR*. In each case, a_3 is significantly less than $2(a_1 + a_2)$. The genes, *GA* and *UR* appear to be about the same distance from their respective centromeres, seven and eight stranes respectively. The mating type locus is located 16 stranes from its centromere. These distances are calculated from the gene-to-gene distances: *UR–GA* = 15, *UR–α* = 24 and *GA–α* = 23.

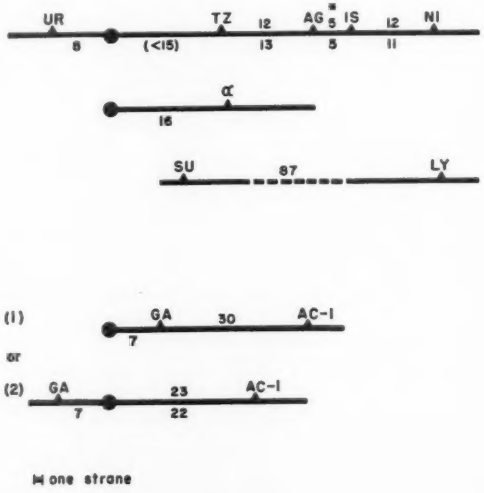
Small linkage groups: L distributions in Table 2 indicate three linkage groups: *SU* and *LY; GA* and *AC-1;* and *UR, IS, AG, TZ* and *NI*.

The linkage of *SU–LY* is the first confirmation of this linkage group, first encountered in family 106. The tetrad distributions in both families are significant (34:10:93, $\chi^2 = 13.09$, $P = 3 \times 10^{-4}$ in family Y-1, while the distribution is 15:2:33, $\chi^2 = 9.99$, $P = 1.5 \times 10^{-3}$ for family 106). Further, there are no tetrad distributions recorded which contradict these L distributions, so that this linkage group appears to be established.

Direct linkage of *AC-1* with *GA* was obtained (74:4:50), and the distance is 29–30 stranes. This confirms previously observed linkages between these genes. Tetrad distributions in other families are:

Family no.	Tetrad distribution of *AC-1* and *GA*		
	I	II	III
87	14	0	4
110	9	3	19
130	44	2	14
131	7	1	5

Since *AC-1* reveals direct linkage with *GA*, an effort was made to place this gene on the *GA* chromosome with respect to the centromere. However, in the absence of a third gene on the same chromosome, the only available distances for this determination are those of the gene-to-centromere-to-gene distances between genes on separate chromosomes. In this family both *UR* and α exhibited F distributions with *AC-1* (Table 2), indicating that the latter *does* lie in the vicinity of a centromere. *AC-1* may be placed on the *GA* chromosome in two arrangements: (1) distal to *GA* on the same chromosome arm or (2) on the opposite arm from *GA*, the centromere being between them. The observed distances *AC-1*—*UR* and *AC-1*—α are 40 and 39 stranes, respectively. When this latter distance is added to the gene-to-centromere distances of *AC-1* and *UR* the total distance required for arrangement (1) of *AC-1* to *UR* is 44–45 and *AC-1* to α is 52–53. Arrangement (2) yields a distance from *AC-1* to *UR* of 30–31 and from *AC-1* to α of 38–39. It is evident that neither arrangement can be preferred since they both contain a similar degree of nonadditivity. That is, $40 \neq 44$–45 while $39 \neq 52$–53 on arrangement (1), and $40 \neq 30$–31 while $39 = 38$–39, on arrangement (2). Arrangement (2) yields a slightly more consistent distance for *AC-1* to *UR* and α than does arrangement (1) (Figure 1).



CHROMOSOME MAPS OF FAMILY Y-1

FIGURE 1.—The above chromosome map shows the four linkage groups discussed in the text. Heavy lines denote chromosomes, dark circles denote centromeres, and triangular points denote the position of loci. Distance is given in stranes (mean number of Poisson exchanges per meiosis) using D_1 above the line and D_2 below the line. The meaning of the asterisk, the distance in the centromere—*TZ* segment, and the preferred order of genes is discussed in the summary and earlier sections.

Linkage in chromosome I: Significant L distributions ($a_1 > a_2$) (SHULT and LINDEGREN 1956) were exhibited for the pairs of loci: *TZ—IS*, *TZ—AG*, *TZ—NI*, *AG—IS*, *IS—NI*, and *AG—NI*. The value of $a_1 - a_2$ is smallest for the genes *TZ—NI* indicating that this is the longest region. This is confirmed by noting that a_3 is also largest for this region. By noting the relative frequencies of $a_1 - a_2$ and a_3 between the other gene pairs the implied order is either (a) *TZ—AG—IS—NI*, or (b) *TZ—IS—AG—NI*. The distances in stranes calculated as D_1 are: 12 for *TZ—AG*, 12 for *TZ—IS*, 5 for *AG—IS*, 12 for *IS—NI*, 14 for *AG—NI* and 28 for the total region *TZ—NI*. Map distances measured in stranes as D_2 are: 13 for *TZ—AG*, 13 for *TZ—IS*, 5 for *AG—IS*, 11 for *IS—NI*, 15 for *AG—NI*, and 30 for the total region *TZ—NI*. Although either of the two suggested orders, (a) and (b) yield good additivity of simple regions for the total region *TZ—NI* on the basis of either D_1 or D_2, the subtriplet, *TZ*, *AG*, *IS*, is nonadditive in either order. (Causes of this nonadditivity are discussed in a later section.) However, *AG—IS—NI* exhibits a more additive relation ($5.5 + 11.6 = 14.0$ for D_1 and $4.8 + 11.2 = 15.2$ for D_2) than *IS—AG—NI* ($5.5 + 14.0 = 11.6$ for D_1 and $4.8 + 15.2 = 11.2$ for D_2). Thus, the data demands a strong preference for order (a) although it should be understood that (b) is a plausible order. The two orders differ only by an interchange of *AG* and *IS*, bounding a short region of five units. In the following analysis, order (a) is assumed for the sake of uniformity. Minor changes in interpretation which result using order (b) are noted when they occur.

It is known from a number of other crosses (DESBOROUGH and LINDEGREN 1959; and in particular (SHULT and DESBOROUGH 1960) that the *IS* locus is located on the right arm of the chromosome carrying *UR* on its left arm. This chromosome has been designated chromosome I. The tetrad distribution for *UR—TZ* (12–0–60) exhibits a significant deviation between the frequencies of the two ditype tetrads ($P < 1\%$). The tetrad distributions for *UR—AG* (21–8–98) and *UR—IS* (24–10–96) exhibit subsignificant deviations between these two frequencies ($P \approx 1.8\%$), while I:II for *UR—NI* (21–20–90) does not deviate from 1:1. The fact that *IS* is located on the same chromosome as *UR* strongly suggests that these linkages are a reality rather than deviant samples. In the tetrad distributions for *UR—TZ*, *UR—AG*, *UR—IS*, and *UR—NI*, the fact that $a_1 - a_2$ decreases monotonically, (16.7%, 10.8% ≈ 10.2%, 0.8%) suggests the linear order, *UR—TZ—(AG, IS)—NI*, consistent with either order (a) or (b) above.

As was noted earlier, both *UR* and *GA* are centromere-linked markers, *UR* being eight stranes from its centromere. It is known from a review of all previous crosses within the Lindegren Breeding Stock (DESBOROUGH and LINDEGREN 1959; LINDEGREN, LINDEGREN, SHULT, and DESBOROUGH 1959) that the centromere of chromosome I is located between *UR* and *IS*, in general *UR* being within 20 units from the centromere. Placement of the centromere between *TZ* and *IS* violates not only the distance of eight stranes from *UR* to its centromere but would imply that *TZ—GA*, *AG—GA* or *IS—GA* exhibit F distributions while *UR* exhibits a T distribution, all of which is contrary to observation. Thus, we must place the centromere between *UR* and *TZ*.

Interference: two-point analysis

Between genes on the same chromosome the frequency of a_3 is expected to increase and approach a limit, .6667, as the distance increases. As noted previously this rule holds between the genes *TZ—AG—IS—NI*. However when *UR* is considered, there is a *descending* sequence of values for a_3 in *UR—TZ*, *UR—AG*, *UR—IS* and *UR—NI*. The percentages of tetratype tetrads are: 83.33, 77.16, 73.84 and 68.70, respectively. This descending sequence is in agreement with what would be expected on order (a) if exchanges in *TZ—AG*, *AG—IS*, and *IS—NI* did not interfere with exchanges in *UR—TZ*, so that all high tetratype frequencies with *UR* are the *consequence* of the inordinately high frequency localized between *UR* and *TZ*. On order (b) the sequence of tetratype frequencies with *UR* would be 83.33, 73.84, 77.16 and 68.70 percent. This order still indicates a high intraregional interference in *UR—TZ*, but requires in addition a slight positive chromosomal interference between exchanges in *UR—IS* and *IS—AG*. Thus, order (a) leads to a simpler hypothesis. This frequency deviates significantly from two thirds with $P < 0.27\%$. On a hypothesis of no interference within the region *UR—TZ*, the expected number of tetratype tetrads is given by a formula equivalent to one originally due to PAPAZIAN (1951) as $\frac{2}{3}(1 - e^{-3\lambda/2})$, where $\lambda = D_1/50$. On this hypothesis, 44.68 tetratype tetrads were expected while 60 (out of 72) were observed. The departure is significant ($\chi^2 = 13.84$, $P < 0.1\%$ at 1 d.f.). Is it possible that this interference effect between *UR* and *TZ* is due entirely to chromatid interference? It has been noted (SHULT and LINDEGREN 1959) that the maximum tetratype frequency cannot exceed 67.56 percent under the effects of chromatid interference *alone*. Therefore, positive intraregional *chromosomal* interference must occur to produce 83.33 percent tetratype tetrads. This may occur either in the presence or absence of chromatid interference expressed as an excess of 3-strand doubles. Some information concerning the possibility of an excess of 3-strand doubles within the region *UR—TZ* may be gained from an examination of chromatid interference in a 3-point analysis of the data. The analysis presented below, indicates, if anything, a *deficiency* in the frequency of 3-strand doubles, suggesting that the interference effect in *UR—TZ* is due entirely to positive chromosomal interference.

Does the positive chromosomal interference, localized in *UR—TZ*, extend *across* the centromere? The tetrad distribution for *GA—TZ* exhibits 80.26 percent tetratype tetrads. (Note that tetratype frequencies for *GA—TZ*, *GA—AG*, *GA—IS*, and *GA—NI* also form a *descending* sequence: namely, 80.26, 76.00, 72.06 and 70.37 percent, respectively. This also indicates that nearly all interference in *GA—*0 0*—TZ—AG—IS—NI* is localized in *GA—*0 0*—TZ*. "0" denotes centromere and supports order (a) on the basis of parsimony.) On the other hand, no such excess occurs in the tetrad distribution for *UR—GA* ($a_3 = 24.1\%$). Thus, the positive chromosomal interference between *UR—TZ* is localized entirely within the centromere-*TZ* segment of chromosome I.

Interference: three-point analysis

The results of ten 3-point analyses on the loci, *UR*, *TZ*, *AG*, *IS* and *NI*, are given in Table 3. The numbers of observed and expected double exchanges are given in the last two columns of Table 3. Because of the agreement of observed and expected values, no chromosomal interference was evident *between* simple regions of chromosome 1. Also, no interregional chromosomal interference appears if order (b) is used in the *TZ—NI* portion of the table.

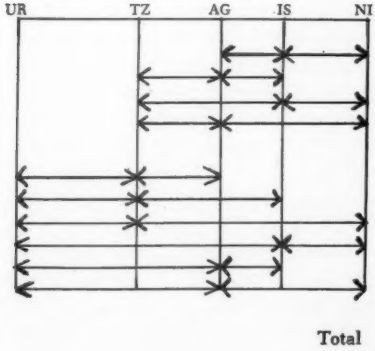
The number of 2-, 3-, and 4-strand double exchanges were also examined in each of the ten 3-point tests. If chromatid interference is absent, these numbers should appear in the ratio 1:2:1. The column marked "P" gives the probability that a random sample of the size observed deviates from 1:2:1 as far, or further, than the observed ratio.

There is evidence of an excess of 2-strand double exchanges in one pair of adjacent simple regions of this chromosome (*AG—IS—NI*). Although the numbers are small, the results are significant on the basis of a binomial distribution of double recombinations. When longer compound regions are considered, this effect is enhanced in the right arm of the chromosome (from *TZ* to *NI*). The total for this portion of the chromosome is 20 2-strand doubles, no 3-strand doubles, and one 4-strand double. This distribution deviates markedly from 1:2:1, with P less than one chance in a million. Other distributions comprising this total are themselves significant, 7:0:1 ($P < 10^{-3}$) and 5:0:0 ($P < 10^{-3}$).

It is important to note that the excess of 2-strand double exchanges cannot be

TABLE 3

Three-point chromatid and chromosomal interference data



Double exchanges 2:3:4	P	Number of doubles Expected	Observed
6: 0: 0	*3.4×10^{-5}	2.62	6
2: 0: 0		.43	2
7: 0: 1	*10^{-3}	4.95	8
5: 0: 0	*10^{-3}	3.68	5
20: 0: 1	$<10^{-6}$		21
2: 6: 2		12.2	10
3: 8: 2		12.46	13
5:10: 7		21.53	22
3: 9:11	3.7%	22.25	23
6: 1: 2	*1.0%	7.96	9
6: 6: 9	8.6%	20.59	21
25:40:33	<0.1%		98
Total 45:40:34	<0.1%		119

"P" indicates the probability that a random sample of 2-, 3- and 4-strand double exchanges of the observed size deviates from 1:2:1 as far or further than the observed distribution. An asterisk indicates the "P" was calculated from a two-dimensional binomial distribution of random variates; absence of an asterisk indicates an approximation of this computation, the standard χ^2 test with two degrees of freedom.

explained on the assumption of faulty gene arrangement. With one exception (order (b)) all other gene arrangements of *TZ*, *AG*, *IS* and *NI* imply an interpretation of the data leading to even higher frequencies of 2-strand doubles. This single exception, *TZ—IS—AG—NI*, although it is a plausible gene arrangement, exhibits a ratio of 2-:3-:4-strand double exchanges of 6:0:0 for simple regions in *TZ—NI* and to a total of 18:0:1 for all regions in *TZ—NI*. Thus, the appearance of strong "negative" chromatid interference cannot be eradicated by changing gene order.

The "negative" chromatid interference also cannot be explained (at least along classical lines) as the result of the faulty use of the assumption that the loci are in a *linear* order. In order to hypothesize a nonlinear arrangement of *TZ*, *AG* and *IS*, one must assume that one of the three genes is one chromosome, the other two on opposite arms of a second. In order to preserve the appearance of linkage, this assumption requires all three genes closely linked to their respective centromeres and strong preferential segregation of these centromeres in parental combination. On this assumption, one could predict a low tetratype frequency ($< 17\%$) between *GA* and at least one member of the triplet *TZ*, *AG*, *IS*. Yet all these frequencies are well above 67 percent (see Table 2), contrary to any such assumption. Thus, the appearance of "negative" chromatid interference cannot be ascribed to nonadditive quasi-linkages. "Quasi-linkage" is the empirical appearance of linkage between genes located on non-homologous chromosome, due to preferential segregation of chromosomal regions or sites, if not the centromeres themselves. See WALLACE (1958) for use of this term, and MICHIE and WALLACE (1953), RHOADES (1942), and LONGLEY (1945) for discussion of phenomena producing this effect.

When the region *UR—TZ* is included (lower six rows of Table 3) the excess of 2-strand double exchanges disappears. This strongly suggests that the highly localized excess of single exchanges, occurring between *TZ* and its centromere, does not engage in the chromatid interference pattern so predominant among exchanges to the right of *TZ*. These single exchanges exhibit a random strand relationship with exchanges in the right portion of this chromosome. The ratio of double exchanges being 25:40:33 (which does not deviate from 1:2:1). Perhaps because of the fact that the excess of 2-strand doubles in *TZ—NI* drains heavily on the frequency of 3-strand doubles, the *total* distribution of strand relationships is slightly deficient in 3-strand doubles. This distribution 45:40:34, deviates from 1:2:1 with $\chi^2 = 14.81$ and $P < 7 \times 10^{-4}$.

A similar conclusion holds when order (b) is used in *TZ—NI*. The total distribution of strand relationships between singles in *UR—TZ* and exchanges in *TZ—NI* is also random (19:40:32). The weak deficiency of 3-strand doubles in the total for all pairs of regions is now subsignificant (the distribution is 37:40:33, $P = 1.5\%$).

DISCUSSION

From the hybrid 24383 × 26466, 140 asci were dissected and tested for 15 Mendelian segregating phenotypes. Of these, all four spores survived in 130

tetrads, while one spore did not survive in each of ten asci. Four new loci, not previously described, were made heterozygous in this hybrid. Of these, three were found to be linked with known loci, while the new adenine mutation, *AD-a*, failed to show any linkage. The standard centromere linkages, previously reported (LINDEGREN 1949b; LINDEGREN and LINDEGREN 1951; HAWTHORNE 1955; LEUPOLD 1956; ROMAN 1956; SHULT and LINDEGREN 1956; LINDEGREN, LINDEGREN, SHULT and DESBOROUGH 1959; DESBOROUGH and LINDEGREN 1959) for α (mating type), *GA* and *UR*, were confirmed in this family. The genes *UR*, *NI*, and *IS*, previously known to be on chromosome I, (LINDEGREN, LINDEGREN, SHULT and DESBOROUGH 1959; DESBOROUGH and LINDEGREN 1959) again appeared in a common linkage group with two of the new loci. The order is *UR*—centromere—*TZ*—(*AG*, *IS*)—*NI*. (*AG*—*IS* is a "short" region of five units; it is not known with certainty which of these loci is closest to *TZ*, although AG is more strongly implicated than *IS*.)

This family exhibited two unusual features of crossing over in this chromosome: (1) the appearance of strong "negative" chromatid interference in the portion of the chromosome distal to *TZ* and (2) an extreme excess of single exchanges localized in the segment centromere—*TZ*. (It was shown that the former effect (1) was genuine in the sense that it is not the result of faulty assignment of gene order or nonadditivity due to quasi-linkage (WALLACE 1958).) It was also proved that the latter (2) is true chromosomal interference rather than the result of an excess of 3-strand doubles.) The localized exchanges in centromere—*TZ* do not appear to engage in the negative chromatid interference pattern described in (1).

Another striking feature of chromosome I in this family is that *NI* is distal to *IS*. This is diametrically opposed to the observations in family 113 (DESBOROUGH and LINDEGREN 1959) where *NI* is *proximal* to *IS*. This difference suggests the possibility that the parents of family Y-1 are homozygous for an inversion containing *IS*, although this hypothesis has not been tested. (In the absence of adequate cytology, two genetic observations are pertinent: (1) drastic reduction in crossing over frequency between *IS* and *NI* in 4-viable-spored tetrads derived from a cross between progeny of 113 × progeny of Y-1, and (2) 2:2 segregation of the "crossover suppressor" in 113 × Y-1 as determined by backcrosses from all members of a 113 × Y-1 tetrad with standard 113 stocks, and standard Y-1 stocks.) An inversion in this region might account for the non random occurrence of crossovers in the segment of the chromosome arm not involved in the inversion, since chromosomal rearrangements have been shown to have definite effects on crossing over (LEWIS 1945).

The chromosome map given in Figure 1 gives a total résumé of the linkage data gleaned from Table 2. The asterisk denotes the fact that the region *AG*—*IS* may actually be reversed so that the order of all five genes is *UR*—*TZ*—*IS*—*AG*—*NI*. (It is shown in the text that this change in order in no way alters the conclusions concerning chromatid and chromosomal interference.) On the assumption that the excess of single exchanges in the segment centromere—*TZ* (see an earlier section) can be represented as a single exchange occurring in 100

percent of the meioses imposed on a population of Poisson-distributed exchanges, the length of this segment is 15 stranes. (Under these assumptions, the appropriate mapping function is $D_s = -33.33\ 1n(3a_s - 2)$ (SHULT and LINDEGREN 1956).) The assumption of lower frequencies for this exchange (which seems more likely in view of the significant linkage between *UR* and *TZ*) lead to shorter distances for this segment, hence, the symbol "(< 15)" in Figure 1. Of the two possible arrangements of the centromere on the *GA—AC-1*—chromosome, order (2) is preferred, although the difference is not significant.

SUMMARY

Both chromatid and chromosomal interference were revealed in chromosome I of Saccharomyces by tetrad analysis of a hybrid heterozygous for five genes (gene order, *UR*—centromere—*TZ*—(*AG*, *IS*)—*NI*). An excess of 2-strand double exchanges (20:0:1) ("negative" chromatid interference) occurred in the portion of the chromosome distal to *TZ*. The excess of 2-strand doubles was not the result of faulty assignment of gene order nor of nonadditivity due to quasi-linkage. An excess of single exchanges was localized in the segment, centromere—*TZ*. The excess of localized single exchanges was not due to an excess of 3-strand doubles in this region. These localized exchanges did not engage in the "negative" chromatid interference pattern in the portion of the chromosome distal to *TZ*.

ACKNOWLEDGMENTS

It is difficult, in assigning authorship to a paper from this laboratory, to give proper credit to all the people who have participated. For example, the current analysis was made possible by the discovery of the gene *TZ* which discriminates between sensitivity and resistance to 1, 2, 4-triazole (the testing of which was suggested by DR. SEYMOUR HUTNER). Discoveries of this type are the outcome of the activities of a group of graduate students working with MRS. LINDEGREN on the breeding stocks under the direction of MRS. DELORES SEKETA. These students currently include MISS ALICE LEE (1960), MISS DONNA SHEUNG HUANG (1960), MR. EDWIN CHENG and MR. CHI HANG LEE. All the nutrilite deficiency and toxicity tests are made by this group and are included in the description of the pedigree. The highly inbred breeding stock carries many factors which tend to reduce its fertility, and the crosses which one would like to make are not always possible. Many tests are usually necessary before a hybrid of sufficient fertility for dissection can be successfully made. This phase of the activity is in MRS. LINDEGREN's hands. She usually also dissects the tetrads. In this instance, the dissection of the tetrads and many of the tests were performed by MR. TADASHI YOSHIDA. Analysis of mating type and fermentation tests are performed by MISS MARCELLA ZINZILIETA and MRS. PRISCILLA GRUBER, students who are supported by the Undergraduate Research Participation Program (N.S.F. 8385).

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SOME BIOCHEMICAL ASPECTS OF THE POSTIRRADIATION MODIFICATION OF ULTRAVIOLET-INDUCED MUTATION FREQUENCY IN BACTERIA[1]

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WITKIN (1956, 1958) has cited considerable evidence which indicates that, in bacteria, ultraviolet light (UV) induced mutation is irreversibly established only after an appreciable delay. Her studies suggest that the induction process is completed during the period prior to the first postirradiation cell division and is dependent on the availability of amino acids. WITKIN suggested that protein synthesis is involved in mutation induction since she found that chloramphenicol (Chl), an antibiotic which blocks protein synthesis, appreciably lowers the mutation frequency obtained with a given dose of UV.

HAAS and DOUDNEY (1957) presented evidence suggesting that the chemical basis for UV-induced mutation involves nucleic acid precursors altered *in vivo* by UV. Later studies (DOUDNEY and HAAS 1958, 1959a) indicate that ribonucleic acid (RNA) synthesis, as well as protein synthesis, is involved in the mutation induction process. These results led to the suggestion that RNA and protein are involved in replication of deoxyribonucleic acid (DNA). As one possibility it was suggested that the RNA is modified through incorporation of the UV-altered purine or pyrimidine and that this modification in RNA specificity leads to a change in the genetic specificity of the DNA subsequently formed.

Recent studies (HAAS and DOUDNEY 1959, 1960) demonstrate that DNA synthesis is the final step in the mutation induction process. These studies further indicate that protein synthesis must follow DNA synthesis for phenotypic expression of the induced mutation. Chl blocks mutation expression when added to the culture immediately following postirradiation synthesis of DNA. It is clear that the *functional* mutated gene is formed with DNA replication.

Although the basis of UV-induced mutation has been investigated intensively during the last decade, a number of points remain to be elucidated before any hypothesis for mechanism can be established with certainty. In the first place, the nature of the initial receptor of the UV energy which leads to mutation must be established. While the data of HAAS and DOUDNEY (1957) is most readily interpreted as indicating the UV chromophore to be nucleic acid precursors present in the cell at the time of irradiation, the alternate hypothesis that the genetic material absorbs the UV which leads to mutation receives widespread

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support. Also in question is the biochemical basis for the reversibility (or prevention) of mutation following UV exposure (i.e., whether the lowering of mutation frequency by various postirradiation treatments is due to an active metabolic event or, conversely, to the physical or chemical "decay" of a "premutational state"). A third factor which must be clarified concerns the nature of the involvement of RNA and protein synthesis in mutation induction and in DNA replication.

The present report describes experiments designed to elucidate the nature of the processes leading to postirradiation decline in induced mutation frequency and the involvement of RNA and protein synthesis in DNA replication and in mutation induction. Efforts to characterize the nature of the mutational chromophore will be described in another report.

MATERIALS AND METHODS

The mutations studied result in reversion of the tryptophan requirement of *Escherichia coli* strain WP2 ($tr^- \rightarrow tr^+$). The mutation assay media used was minimal agar medium supplemented with 2.5 percent Difco nutrient broth (DOUDNEY and HAAS 1958). The low level of amino acids supplied by the nutrient broth allows a somewhat limited degree of mutation induction, and the tryptophan present permits the unreverted tr^- cells to make enough divisions to develop small, but visible, colonies at proper dilutions, and also enables mutation expression in the reverted tr^+ cells. The techniques followed have been previously described (HAAS and DOUDNEY 1957; DOUDNEY and HAAS 1958, 1959a). Briefly they are as follows. The culture is grown for 15.5 hours at 37°C with aeration in 50 ml portions of minimal medium supplemented with 0.2 mg per ml of DL-tryptophan. It is then held at 6°C for one hour, the cells centrifuged down in the cold, and resuspended in fresh medium at 37°C. This culture is grown for an additional period of 50 minutes, then chilled to halt further growth. The cells are centrifuged down again, washed once in cold 0.9 percent saline, and finally resuspended in cold minimal medium. Unirradiated suspensions are adjusted to contain about 6×10^8 colony-forming organisms per ml. UV irradiation is for ten seconds at a distance of 30 cm (964 ergs per mm^2). After UV exposure the sample is diluted one to four into appropriately supplemented growth medium and incubated for the indicated time interval at 37°C on a reciprocal shaker prior to plating at appropriate dilutions onto mutation assay agar medium. The plates are incubated for three days at 37°C and scored for total survivors (tr^-) and induced revertants (tr^+). The usual precautions to prevent photoreversal are observed subsequent to UV exposure.

Culture samples, taken at appropriate intervals during the postirradiation incubation period for analysis of RNA, DNA and protein, are precipitated and washed with 0.5 N perchloric acid. Nucleic acids are hydrolyzed by incubation in perchloric acid for 50 minutes at 70°C (OGUR and ROSEN 1950). Analysis for DNA is that of BURTON (1956). For RNA determination, the ultraviolet absorption at 260 mμ and 290 mμ is determined (VISSER and CHARGAFF 1948), and the

amount of DNA, as determined by the Burton analysis of the same sample, is then subtracted with correction for extinction coefficients. Protein is determined by the Folin method (LOWRY, ROSEBROUGH and RANDALL 1951).

EXPERIMENTAL RESULTS

Mutation frequency decline and macromolecular synthesis: The concept has been advanced that the level of mutation frequency induced by a given UV dose is related to the timing of DNA synthesis following irradiation. One investigator (WITKIN 1956, 1958) proposed that delay in DNA synthesis would increase mutation since more time would be available for protein synthesis, presumably involved in mutation induction. Others proposed that delay in DNA synthesis would decrease mutation since more time would be available for a hypothetical unstable "premutational" state to revert to a state not leading to mutation induction (KIMBALL, GAITHER and WILSON 1959). Both points of view imply that DNA synthesis is the terminal event in mutation induction.

Previous work has demonstrated that UV does cause a delay in DNA synthesis (KELNER 1953). Recently, several workers have shown that resumption of DNA synthesis following UV exposure requires prior synthesis of RNA and protein (HAROLD and ZIPORIN 1958; DOUDNEY 1959; DRACULIC and ERRERA 1959). Incubation with inhibitors blocking either RNA or protein synthesis, prior to initiation of DNA synthesis, leads to an additional lag in DNA synthesis during the time the necessary RNA and protein is formed. Many of the agents and conditions which block RNA or protein synthesis also promote a marked lowering in the expected induced mutation frequency. Whether the mutation frequency decline promoted by these agents can be ascribed to delay in DNA synthesis, or to an active decline process promoted by the inhibitors and independent of delay in DNA synthesis, remains to be resolved. If a quantitative correlation can be established between the effect of these agents on mutation frequency and their effect in delaying DNA synthesis, then the hypothesis suggested by KIMBALL and co-workers will receive considerable support. However, if decline in mutation frequency is independent of the effect on DNA synthesis, the hypothesis that mutation frequency decline is an active and enzymatically conditioned process (DOUDNEY and HAAS 1958) will appear more plausible.

Results of experiments designed to differentiate between the alternatives posed above are presented in Figures 1, 2 and 3, and in Table 1. These experiments measure the effect of increasing periods of immediate postirradiation treatments which block RNA or protein synthesis on the mutation frequency and on the subsequent synthesis of DNA in the culture. The mutation frequency is assayed in this case when the relative amount of DNA has just doubled. However, it makes little difference when the sample is plated *after* the indicated interval of treatment since the mutation frequency observed upon plating remains at approximately the same level until the first cellular division (usually after 100 minutes incubation). Figure 1 demonstrates the effect of 10, 20 and 25 minutes incubation in the presence of the uracil analogue, 6-aza uracil. This analogue has

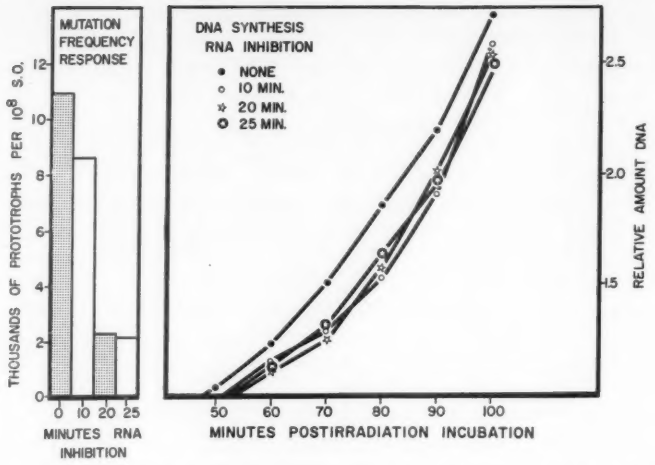

FIGURE 1.—Effect on yield of prototrophs, and on subsequent DNA synthesis in *Escherichia coli* strain WP2 (tryptophan requiring), of incubation with 6-aza uracil (5 mg per 100 ml medium) for varying time intervals following UV irradiation (*RNA inhibition*). The 6-aza uracil inhibition was reversed after the indicated period of incubation in liquid growth medium (minimal medium + casein hydrolysate, 2 mg per ml and 0.05 mg per ml DL-tryptophan) + 6 AU by the addition of uridine (5 mg per 100 ml). The increase in DNA relative to unincubated controls was followed during subsequent incubation. The times given are from the start of incubation following UV irradiation, and include both incubation periods (+ 6 AU and after reversal with uridine). Mutation frequency was assayed by plating after 80-minutes incubation, and prior to cell division (at 90–100 minutes), on nutrient broth supplemented mutation assay agar. Mutation frequency does not vary with plating from 20–90 minutes. No effect of 6 AU on survival was noted.

been shown to block RNA and protein synthesis and also to promote mutation frequency decline (DOUDNEY and HAAS 1959b). After the indicated intervals of incubation the effect of 6-aza uracil was reversed by the addition of uracil. Figure 2 demonstrates the effect of incubation of the culture for 10, 15, 20 and 25 minutes in the absence of nitrogen. This treatment also promotes rapid mutation frequency decline (DOUDNEY and HAAS 1958). After the indicated period of incubation, a mixture of amino acids is added to the medium. Figure 3 demonstrates the effect of depriving this tryptophan-requiring auxotrophic strain of tryptophan for 10, 20 and 25 minutes before adding the amino acid. Similar experiments carried out with chloramphenicol addition (Table 1), and with the addition of the amino acid analogue, β-thienylalanine (results not given), show comparable results. In the case of Chl addition, the cells were removed from the Chl by rapid centrifugation, washed and resuspended in fresh Chl-free medium.

These experiments demonstrate that a wide variety of conditions and agents which lead to lower mutation frequency through blockage of RNA or protein synthesis during the immediate postirradiation incubation period produce little

or no effect on subsequent DNA synthesis. There is a very slight delay in DNA synthesis in some experiments which seems to occur with "short-period" (five minute) holding of irradiated cultures under conditions where RNA or protein synthesis cannot take place. However, this delay is not correlated with the greater proportion of the mutation frequency decline observed. Only with treatment of at least 20 minutes duration is the maximum amount of decline in mutation frequency obtained. This decline is not associated with any further change in the pattern of DNA synthesis.

Similar results are observed for RNA and protein synthesis in the culture. Net RNA synthesis begins only after 25–30-minute postirradiation incubation, and net protein synthesis after 30-minute incubation. Treatments during the first 20 minutes which promote maximum mutation frequency decline do not appreciably modify the subsequent synthesis of RNA and protein by the culture.

Dinitrophenol (DNP), which interferes with oxidative phosphorylation, has been shown to prevent decline in mutation frequency (Haas and Doudney 1958; Doudney and Haas 1959a). This suggests that the decline process depends on energy yielding transformations. Table 1 demonstrates that, while the decline promoted by Chl is prevented by the addition of DNP, there is no effect of DNP on subsequent DNA synthesis. DNP interference with the decline in mutation

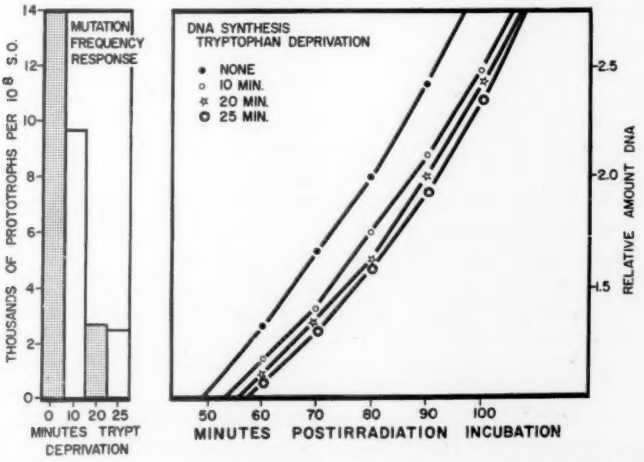

Figure 2.—Effect on yield of induced prototrophs, and on subsequent DNA synthesis in *Escherichia coli* strain WP2 (tryptophan requiring) of incubation without tryptophan for varying time intervals following UV irradiation. The incubation medium was minimal plus a mixture of 19 amino acids excluding tryptophan. DL-tryptophan was added (0.05 mg per ml) after the indicated intervals of incubation. The times given are from the start of incubation following UV irradiation, and include the incubation periods both without and with tryptophan. Mutation frequency was assayed by plating after 80-minutes incubation and prior to cell division (at 90–100 minutes) on nutrient broth supplemented mutation assay agar. Mutation frequency does not vary with plating from 20–90 minutes. No effect of tryptophan deprivation on survival was noted.

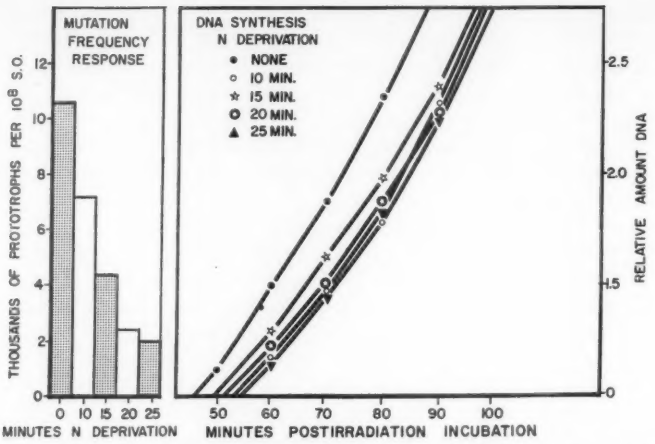

FIGURE 3.—Effect on yield of prototrophs, and on subsequent DNA synthesis, in *Escherichia coli* strain WP2 (tryptophan requiring) of incubation in nitrogen-free medium (minimal medium from which the ammonium sulfate was omitted) for varying time intervals following UV irradiation. Enzymatically digested casein hydrolysate (2 mg per ml) and DL-tryptophan (0.05 mg per ml) were added after the indicated interval of incubation. The increase in DNA relative to unincubated controls was then followed during subsequent incubation. The times given are from the start of incubation following UV irradiation, and include the incubation periods both without and with amino acids. Mutation frequency was assayed by plating after 80-minutes incubation and prior to cell division (at 90–100 minutes) on nutrient broth supplemented mutation assay agar. The mutation frequency does not vary with plating from 20–90 minutes. No effect of nitrogen deprivation on survival was observed.

frequency promoted by the other treatments gave similar results as to DNA synthesis. It therefore appears possible to selectively interfere with the decline process without modifying subsequent DNA synthesis.

From the experimental results reported above it is evident that the delay in DNA synthesis following UV exposure, as observed by HAROLD and ZIPORIN (1958), DOUDNEY (1959) and DRACULIC and ERRERA (1959) when RNA or protein synthesis is blocked, is not related to the mutation frequency decline observed. In fact, as will be discussed later, the delay in DNA synthesis, as observed by these investigators, appears only when *net* RNA or protein synthesis in the culture is interrupted (after 25–30-minute incubation); the RNA and protein requisite to DNA synthesis appears to be formed with the initial *doubling* of RNA in the culture. It is quite clear that delay in DNA synthesis cannot be held responsible for reducing the induced mutation frequency following UV irradiation.

Mutation fixation and mutation frequency decline: Since it has been shown that susceptibility of the "potential mutation" to treatments leading to lower mutation frequency is lost with increasing periods of postirradiation incubation (WITKIN 1956; DOUDNEY and HAAS 1958), it is apparent that the blockage to

RNA or protein formation leads to decline in mutation frequency *only* provided some significant metabolic event has not taken place. This event we have termed "mutation fixation". We define mutation fixation specifically, though arbitrarily, as that biosynthetic event which removes the potential mutation from susceptibility to all treatments which promote this *specific* decline process. We wish to make it quite clear that we do not regard mutation fixation as the final step in the induction of mutation; rather, the final step unquestionably appears to be the initial postirradiation synthesis of the genetic complement of DNA. The fact that certain drastic treatments (i.e., incubation in saline [LIEB 1960]) interfere with mutation induction after the event, defined as mutation fixation, has occurred does not invalidate this concept. Incubation in saline leads to extensive reorganization of cellular metabolism, including breakdown of RNA and requirements for RNA and protein synthesis before DNA synthesis can take place (DOUDNEY and HAAS, unpublished data). It is therefore not surprising that the "potential mutation" may be abolished by such treatments prior to DNA replication and establishment of the mutation in the genome.

A correlation between RNA synthesis and mutation fixation in UV-irradiated cultures has been described previously (DOUDNEY and HAAS 1959a). The postirradiation period required for the progression of mutation fixation in the culture can be followed by addition of Chl after varying postirradiation incubation intervals. Incubation in the presence of Chl is of sufficient duration to permit "complete" mutation frequency decline to take place where the mutation has not been "fixed"; those potential mutations which are fixed will not be affected. This Chl

TABLE 1

Effect of dinitrophenol on "mutation frequency decline" promoted by chloramphenicol and on subsequent DNA synthesis

Minutes with Chl	DNP present	tr^+ cells per 10^8 tr^- cells	Relative amount DNA after incubation Minutes of incubation				
			45	60	75	90	105
0	—	112	1.1	1.5	2.1	2.7	3.5
	+	108	1.1	1.4	1.9	2.6	3.5
10	—	75	1.2	1.5	2.1	2.7	3.6
	+	102	1.1	1.5	1.9	2.3	2.9
15	—	40	1.1	1.4	2.0	2.8	3.5
	+	115	1.2	1.5	1.9	2.5	3.2
20	—	20	1.1	1.5	2.1	2.6	3.6
	+	104	1.1	1.4	1.9	2.4	3.3
25	—	16	1.2	1.5	2.0	2.6	3.3
	+	112	1.2	1.4	2.1	2.5	3.2

After UV exposure, the bacteria were suspended in liquid growth medium (minimal + casein hydrolysate, 2 mg per ml and 0.05 mg per ml DL-tryptophan) containing 20 μg per ml chloramphenicol and ± 5×10^{-3} M dinitrophenol. Following the indicated periods of incubation at 37°C, samples of the cultures were rapidly centrifuged out of this medium, resuspended in fresh growth medium without Chl or DNP and incubation continued. All times given are measured from the start of incubation after UV exposure.

Cell division occurred after 80-minutes incubation in all samples. After the indicated treatment and prior to cell division, the mutation frequency upon plating does not vary significantly. Mutation frequency values given were obtained by plating on nutrient broth supplemented agar medium after 75-minutes incubation. DNA increase is relative to unincubated control. UV delayed DNA synthesis for 45 minutes in this experiment. No effect of Chl or DNP on survival was observed.

"challenge" technique demonstrates, therefore, that fraction of potential mutations which are irreversibly "fixed" at any given interval of incubation following UV irradiation.

LIEB (1960) has questioned the validity of the mutation fixation concept, since she regards the amount of DNA formed during the Chl challenge period as significant in determining the level of induced mutation frequency observed. However, the level of mutation fixation can be related to postirradiation RNA formation under conditions where both RNA and DNA synthesis, as well as protein synthesis, are blocked during "challenge". The uracil analogue, 6-aza uracil, blocks net RNA, DNA and protein synthesis immediately after addition to the culture. Following UV irradiation, short-term incubation with this analogue produces rapid mutation frequency decline which is correlated with that produced by Chl (DOUDNEY and HAAS 1959b). As demonstrated (Figure 1), incubation with this analogue for a time period sufficient for maximum mutation frequency decline does not modify significantly the timing of subsequent DNA synthesis. Table 2 demonstrates that mutation fixation, measured by challenge with 6-aza uracil, is correlated with RNA synthesis as is mutation fixation when determined by Chl challenge. It is evident, therefore, that RNA synthesized after Chl addition has no role in mutation induction. These results would be expected only if *both* RNA and protein synthesis are involved in mutation fixation. Blockage of protein synthesis with Chl would prevent mutation fixation but would allow RNA synthesis to proceed. It is also evident that DNA synthesis during the challenge period is not involved in determining the level of mutation frequency since DNA synthesis does not occur in the presence of 6-aza uracil. Although DNA synthesis does occur in the presence of Chl, the level of mutation induction observed at a given time of Chl addition is identical with that obtained at the same time with 6-aza uracil challenge where no DNA synthesis occurs. Furthermore, the synthesis of DNA in the presence of Chl may be prevented with no modification in mutation frequency by addition of 6-aza uracil after 20-minute

TABLE 2

Relation of macromolecular synthesis to "mutation fixation" as measured by chloramphenicol and 6-aza uracil "challenge"

Minutes incubation prior to challenge	tr^+ cells per 10^8 tr^- cells		Relative amount macromolecule at challenge		
	6 AU challenge	Chl challenge	RNA	Protein	DNA
0	15.3	19.2	1.0	1.0	1.0
15	16.8	14.2	1.0	1.0	1.0
30	35.0	35.3	1.3	1.2	1.0
45	60.0	65.3	1.6	1.6	1.1
60	98.0	106.2	2.1	1.8	1.5
75	90.0	95.9	2.7	2.1	1.9

Following irradiation with UV, the bacteria were suspended in liquid growth medium and incubated at 37°C. At the indicated times either 6-aza uracil (5 mg per 100 ml) or chloramphenicol (20 μg per ml) was added to separate samples and incubation continued for 45 minutes before plating on nutrient broth supplemented agar medium. The RNA, protein and DNA content at the time of antimetabolite addition is given relative to unincubated controls. Cell division occurred after 85-minutes incubation. No effect of 6 AU or Chl on survival was observed in this experiment.

incubation in Chl. These results make it appear improbable that DNA synthesis in the presence of Chl is the basic factor influencing the level of mutation fixation, as contended by LIEB (1960).

An observation which should be emphasized in this regard is that, once the cells have been incubated in the presence of Chl for the minimum time necessary for the decline in mutation frequency to take place, additional incubation in the presence of this inhibitor does not modify the mutation frequency response (Table 3). The decline process is complete to the minimum level attainable within 30-minute incubation in Chl. It is thus evident that the observed number of "potential mutations" sensitive to Chl challenge decreases with the postirradiation incubation, not because of a decrease in rate of the mutation frequency decline process or other factors, but because the process of mutation fixation has rendered a portion of the potential mutations insensitive. The same arguments hold for comparable results observed with 6-aza uracil challenge.

It is evident that if RNA and protein are involved in DNA replication and in mutation fixation (DOUDNEY and HAAS 1958, 1959a), then addition of agents blocking RNA or protein synthesis at a given time of postirradiation incubation would block mutation fixation. Furthermore, DNA synthesis should be blocked where the RNA or protein necessary for DNA replication has not yet been formed. This hypothesis can be tested by use of chloramphenicol since this antibiotic blocks protein synthesis specifically and not DNA synthesis in cells possessing the complete DNA synthesizing mechanism (DOUDNEY 1960). Table 4 demonstrates that, at any given time of Chl addition following irradiation, the capacity of the culture to synthesize DNA without further protein synthesis is related to the amount of postirradiation RNA formed before the antibiotic is added. The culture attains the maximum rate of DNA synthesis in the presence of Chl when RNA has doubled prior to Chl addition. Furthermore, it is evident, from the same data, that a relation exists between mutation fixation and the amount of postirradiation RNA formed. This suggests that mutation

TABLE 3

Mutation frequency decline produced by chloramphenicol added after various postirradiation incubation periods

Minutes incubation prior to Chl addition	tr^+ cells per 10^9 tr^- cells after incubation in Chl Minutes in Chl							
	0	10	15	20	30	40	50	60
0	112.9	55.0	35.0	30.6	19.6	16.4	17.8	16.5
15	106.8	56.8	34.0	29.6	18.2	16.4	17.8	18.7
30	119.9	62.3	52.1	37.8	28.5	29.8	31.7	30.6
40	115.9	81.3	66.6	41.6	36.5	40.7	39.2	38.6
50	111.3	100.6	84.0	87.6	88.0	80.1	76.3	82.1
60	117.6	121.7	107.9	107.6	112.4	118.7	109.8	111.3

After UV irradiation, the bacteria were suspended in liquid growth medium and incubated at 37°C. Chloramphenicol (20 μg per ml) was added to separate samples at the indicated times of incubation, and the samples incubated in the presence of Chl for the times indicated before plating onto nutrient broth supplemented mutation assay agar. No effect of Chl on survival was observed.

TABLE 4

Relation between "mutation fixation", amount of RNA synthesized at chloramphenicol addition and subsequent rate of DNA synthesis in presence of chloramphenicol

Minutes incubation prior to Chl addition	Mutation fixation		RNA		DNA synthesis in Chl				
					Relative amount DNA Minutes incubation				
	tr+ cells per 10^9 *tr*− cells	Percent of max. frequency	Relative amount	Percent increase	60	70	80	90	Percent of max. rate in Chl
0	28.9	16.7	1.00	0	1.06	1.02	1.08	1.00	0
20	23.7	13.6	1.03	3	1.01	1.03	1.04	1.01	0
25	28.8	16.6	1.06	6	1.10	1.12	1.16	1.20	11
30	38.4	22.2	1.17	17	1.12	1.19	1.24	1.33	20
35	45.3	26.1	1.24	24	1.16	1.23	1.32	1.40	28
40	57.0	32.9	1.36	36	1.18	1.24	1.43	1.53	40
45	71.3	41.1	1.48	48	1.18	1.32	1.52	1.71	53
50	106.6	61.3	1.63	63	1.18	1.38	1.58	1.77	62
55	135.0	79.0	1.81	81	1.20	1.45	1.74	2.04	81
60	162.0	93.6	2.02	102	1.23	1.55	1.87	2.06	97
65	169.8	97.6	2.37	137	1.23	1.61	1.89	2.10	100

After UV irradiation the bacteria were diluted into liquid growth medium and incubated. At the indicated times, chloramphenicol was added to separate samples and incubation continued. RNA was determined at the time of Chl addition. Mutation fixation was determined by incubation with Chl for an additional 45 minutes (a period sufficient for complete Chl-promoted "mutation frequency decline") and then plating onto nutrient broth supplemented mutation assay agar. Relative increase in DNA was followed during incubation in Chl. The times given are measured from start of incubation following UV exposure. The *rates* of DNA synthesis were calculated for the period between 60 and 90 minutes, and the percent of the maximum rate in Chl then calculated. DNA synthesis in Chl is linear. The maximum rate of DNA synthesis in Chl was observed with 65-minutes incubation prior to Chl addition. No effect of Chl on survival was observed.

fixation requires the formation of RNA and protein made necessary for DNA replication by the UV irradiation. The data suggest that, if the necessary RNA and protein is formed prior to Chl addition, mutation frequency decline does not take place. The process of mutation fixation therefore appears to be related to the capacity of the cells to synthesize DNA rather than DNA synthesis *per se*.

Two alternative possibilities appear plausible for the involvement of RNA and protein in DNA replication based on the varying distribution of capacity for DNA synthesis in the presence of Chl among cells in the culture with incubation. On the one hand, the progressive acquisition of capacity of the culture to form DNA may be a rapid "all or none" phenomenon in each cell and based on the distribution of the requisite RNA and protein formation in time. According to this hypothesis, formation of RNA and protein necessary for synthesis of the total DNA complement of the cell must occur prior to Chl addition if DNA synthesis is to occur in that cell at all. For example, this might be the case if the RNA is involved in formation of an enzyme system required in DNA replication. To explain the kinetics of DNA synthesis according to this hypothesis, it would be necessary to assume that the time required for formation of the enzyme system in a given cell occupies only a small fraction of the time required for initial doubling of the RNA in the culture and that once the necessary enzyme is formed, that cell is capable of DNA synthesis at maximum rate. On the other hand, progressive gain in competence to synthesize DNA *in each cell* could be correlated with doubling of RNA in the culture, and it could be postulated that

the progressive formation of a rate-limiting enzyme system critical for DNA synthesis in the cell is involved.

If we adopt the point of view that the RNA and protein involved in DNA replication functions, not enzymatically, but directly in DNA synthesis (HAAS and DOUDNEY 1957; DOUDNEY and HAAS 1958, 1959a), then considerations similar to the above would apply. Each cell might rapidly synthesize the total RNA and protein involved, such synthesis requiring only a small part of the time needed for RNA doubling in the culture. Alternately, the RNA and protein required for DNA replication may be synthesized over the entire period required for doubling of RNA in the culture. The latter possibility has an intriguing implication. Partial formation of the RNA involved in DNA replication prior to Chl addition might lead *only* to replication of that part of the genome which corresponds genetically to the partially synthesized RNA. In such a case mutation frequency decline would take place only for that part of the genome which is not replicated, due to incomplete RNA formation. This suggests the possibility (which should be amenable to test) of "mapping" the genes along the length of the RNA as well as the DNA by this technique. This mapping would be based on *differential* mutation fixation of different prototrophic characters in a multiple-requiring auxotrophic strain. WITKIN (personal communication) has found evidence from preliminary experiments which indicates that mapping of genes along the replicating bacterial genome can be accomplished by the Chl challenge technique.

Reduction in mutation frequency by use of purine and pyrimidine analogues: Several purine and pyrimidine analogues have been shown to promote a decline in mutation frequency, but only after a considerable period of incubation (DOUDNEY and HAAS 1959b). Among these analogues are 5-hydroxyuridine (5-HU) and 6-mercaptopurine. The decline promoted by these analogues is correlated with doubling of RNA in their presence. It appears likely that they exert their effect through incorporation into RNA. The analogues do not block RNA synthesis, but convert synthesis from an "exponential" rate to a "linear" rate. This appears to be typical of those analogues incorporated into RNA or protein and interfering with macromolecular function. These analogues which do not block RNA synthesis do not promote the immediate mutation frequency decline *process*, as do analogues which are not incorporated, but are capable of blocking synthesis, i.e., 6-aza uracil (DOUDNEY and HAAS 1959b). We have suggested that the lower mutation frequency promoted by 5-HU is due to synthesis of "nonfunctional" or "rejected" RNA produced by analogue incorporation (DOUDNEY and HAAS 1959a). The mutagenic substrate (i.e., the UV-modified purine or pyrimidine) would be used up in the synthesis of this RNA according to this hypothesis.

Incubation in the presence of a mixture of amino acids immediately following UV exposure markedly increases the mutation yield (WITKIN 1956; HAAS and DOUDNEY 1957; DOUDNEY and HAAS 1958). The time interval required for amino acids to exert a measurable effect was shown by incubating the irradiated bacteria on a high level of amino acids for increasing intervals and then plating

on minimal medium containing a concentration of amino acids which is limiting to mutation frequency (DOUDNEY and HAAS 1959a). Such experiments established that amino acids sufficient to increase to maximum the induced mutation frequency are taken into the cell within 30-minute postirradiation incubation and prior to any measurable protein synthesis. Incubation in the presence of high levels of amino acids does not change the course of subsequent net protein synthesis, nor modify mutation fixation, although such treatment does prevent mutation frequency decline. Therefore, the amino acids taken into the cell must actually "do something" to increase mutation frequency response. We have termed this event "mutation stabilization" for convenience in reference. As one possibility it was suggested that the amino acids interact with nucleic acid precursors so as to "stabilize" temporarily the precursors (including the UV-modified precursors) for subsequent nucleic acid synthesis (DOUDNEY and HAAS 1959a). The possible involvement of nucleotide-amino acid complexes in both nucleic acid and protein synthesis has been previously suggested. However, it seems probable that "mutation stabilization" involves more than formation of amino acid-nucleotide complexes since the blockage of RNA or protein synthesis does not prevent formation of such complexes but does lead to mutation frequency decline. Since no net RNA or protein synthesis can be detected during this period, it may be that RNA and protein synthesis involved in mutation stabilization is quantitatively minor.

The timing of the loss of ability of 5-HU to reduce the level of mutation frequency has considerable bearing on our insight into the nature of the "premutational" state following UV irradiation. If loss of susceptibility to 5-HU promoted decline (which is found correlated with initial doubling of RNA) is correlated with the mutation stabilization process which occurs considerably earlier, then the data would suggest that, in order to reduce mutation frequency, the 5-HU must interact with amino acids in the same way as the hypothetical mutagenic nucleic acid precursors in mutation stabilization.

Figure 4 shows that the effect of 5-HU in lowering mutation frequency is lost in close correlation with the time course of mutation stabilization; 5-HU added after 30-minute incubation has no effect on the mutation yield. These data are in line with the hypothesis that the "potential mutation" exists initially as a modified nucleic acid precursor, and that both the modified precursor and 5-HU must undergo similar interactions with amino acids in order to exert their respective effects. On this basis, incorporation of the modified precursor leads to mutation, while incorporation of 5-HU leads to production of biologically ineffective RNA.

Other experiments were carried out with the objective of determining whether or not incubation with 5-HU for a time period sufficient to attain the lowest mutation frequency possible with this analogue would interefere with subsequent DNA synthesis. Such interference would be expected if the mechanism of 5-HU action in promoting the lower mutation frequency were, for example, formation of a defective enzyme incapable of functioning in DNA synthesis. The results of such experiments (Table 5) show that this hypothesis must be rejected. While

5-HU reduces mutation frequency in correlation with RNA synthesis, no significant delay in DNA synthesis is observed when the analogue is counteracted by addition of uridine at initiation of DNA synthesis. The RNA and protein formed in the presence of 5-HU must therefore be adequate for DNA synthesis, but inadequate for mutation induction. All enzymes required for DNA synthesis

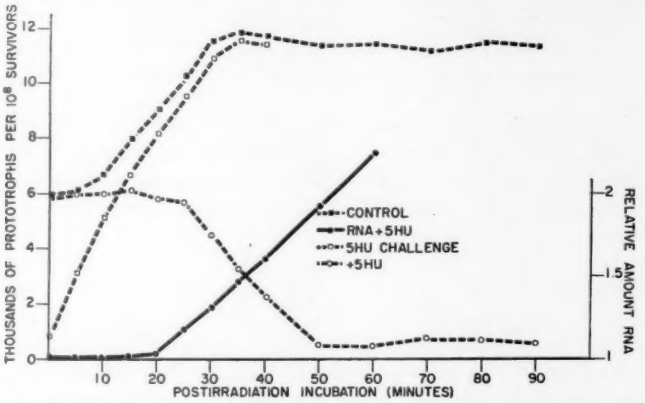

FIGURE 4.—Loss of effectiveness of 5-hydroxyuridine in decreasing mutation yield with incubation following UV irradiation. The control curve demonstrates the mutation frequency observed with plating on nutrient broth supplemented mutation assay agar after the indicated time of incubation in minimal medium supplemented with casein hydrolysate (2 mg per ml) and DL-tryptophan (0.05 mg per ml). The + 5HU curve demonstrates the effect on mutation frequency of incubation in this same medium supplemented with 5-hydroxyuridine (5 mg per 100 ml). The RNA + 5HU curve demonstrate relative RNA increase in the presence of 5-hydroxyuridine during the same period. The 5HU challenge curve demonstrates the effect upon mutation frequency of addition of 5-hydroxyuridine at the indicated time (rather than zero time), and incubating to the 90-minute postirradiation incubation time prior to plating. No effect of 5-hydroxyuridine on survival was noted.

TABLE 5

Effect of incubation with 5-hydroxyuridine on mutation yield and on DNA synthesis

Incubation time (minutes)	tr^+ cells per 10^8 tr^- cells			DNA (relative amount)		
	Control	5-HU	5-HU, uridine added at 60 min	Control	5-HU	5-HU, uridine added at 60 min
0	62.0	66.0	65.8	1.0	1.0	1.0
20	65.6	57.5	59.3	1.0	1.0	1.0
40	68.9	31.5	33.6	1.0	1.1	1.0
60	64.3	18.2	16.8	1.3	1.2	1.2
80	70.1	15.7	15.2	1.9	1.3	1.6
100	53.8	18.6	15.1	2.6	1.4	2.3

After UV irradiation the bacteria were suspended in growth medium ± 5-hydroxyuridine (5 mg per 100 ml). The mutation frequency and increase in DNA relative to unincubated controls was determined for the indicated times of incubation. To an identical set of 5-HU containing samples, uridine (5 mg per 100 ml) was added at 60 minutes incubation to counter the analogue. No effect of 5-HU on survival was observed.

either must have been present following UV exposure or were formed in the presence of 5-HU. The hypothesis that the RNA required is intimately involved in transfer of genetic information fits the 5-HU data since introduction of 5-HU into this RNA could lead to defective RNA. It would be presumed that, in turn, defective DNA unable to perform its obligatory genetic role would be synthesized as a consequence of the defective RNA; the cell would then revert to the "old" DNA for its genetic information. If this is true, the expected mutation yield would not be observed even though RNA and DNA synthesis took place as usual. Only through further experimentation can this possibility be established or rejected.

DNA synthesis in the mutation induction process: We have previously reported experiments which strongly suggest that DNA synthesis is the terminal event in mutation induction (HAAS and DOUDNEY 1959, 1960). These experiments also indicate that DNA synthesis must be followed by a period of protein synthesis if the induced mutations (reversion of the tryptophan requirement in this case) are to be expressed. It seems quite probable that, in these experiments with strain WP2, we are dealing with the initial action of the reverted gene and that the increase in mutation frequency observed on minimal agar after increasing periods of incubation in complete medium is due to the appearance of the missing enzyme involved in tryptophan synthesis which is controlled by the mutated gene. The most reasonable explanation for the results of the experiments is that the final step in the process of UV-induced mutation requires synthesis of DNA but that the mutation cannot express itself phenotypically until the gene has had time to establish the production of the required enzyme.

We have recently carried out experiments with thymine- and amino acid-requiring double auxotrophs of *E. coli* in which the mutation followed was reversion of the amino acid requirement. It was found that absence of thymine prevents mutation expression in these strains but does not lead to mutation frequency decline as determined by subsequent plating onto amino acid- and thymine-containing medium. These results appear to be a direct demonstration of the necessity for DNA synthesis in mutation induction. It is quite clear from the experiments that delay in DNA synthesis due to lack of thymine does not modify mutation frequency in the absence of the conditions which promote the active decline process. These studies of mutation induction in thymine-requiring strains of *E. coli* will be fully described in a separate communication.

The reality of the relation of mutation induction to DNA synthesis may be assessed by testing for the photoreversibility of the mutation after DNA synthesis. One would not expect that the "potential mutation" could be photoreversed after final incorporation of the "genetic change" into the DNA. A number of workers using various mutational systems and organisms have reported that photoreversibility of UV-induced mutation is lost with postirradiation incubation (KELNER 1949; NEWCOMBE and WHITEHEAD 1951; NOVICK and SZILLARD 1949; MATNEY, SHANKEL and WYSS 1958). A similar effect has been reported from studies of the tryptophan-requirement reversion in *E. coli* strain WP2 (DOUDNEY and HAAS 1959a). Figure 5 demonstrates that, for the major

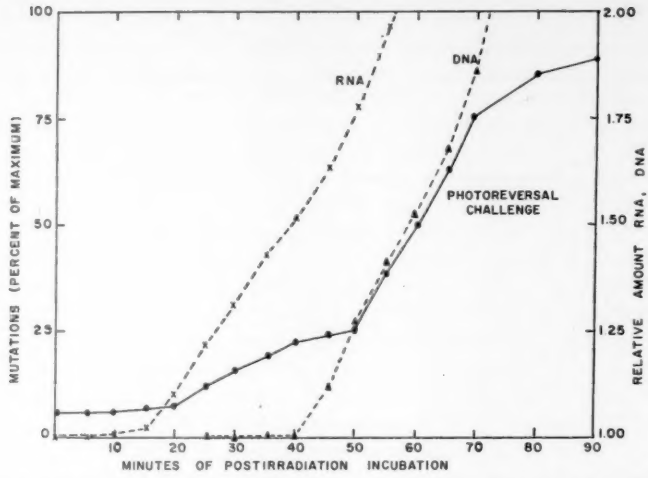

FIGURE 5.—Relation to RNA and DNA synthesis of loss of susceptibility of "potential mutations" to photoreversal with postirradiation incubation. At the indicated times of incubation in liquid growth medium, samples of the culture were chilled and held in ice bath at 2°C either in photoreversing light (two General Electric 500 watt projection bulbs at a distance of six inches) or in the dark for one hour (a period of white light exposure giving maximum photoreversal) prior to plating on nutrient broth supplemented mutation assay agar. At the same times RNA and DNA relative to unincubated controls were determined with identical samples. Maximum mutation frequency observed for control cells (no photoreversal) was approximately 90 prototrophs per 10^6 surviving organisms. Survival following this dose of UV was approximately 50 percent. The white light treatment resulted in photoreactivation to the 70 percent level of survival when applied without incubation. This effect was not apparent after 20-minutes incubation.

portion of the reversions induced, loss of photoreversibility is correlated with the progression of DNA synthesis. Loss of photoreversibility occurs at a rapid rate with initiation of DNA synthesis; at the time that DNA has doubled approximately 85 percent of the potential mutations are no longer photoreversible. The initial loss of photoreversibility, occurring at 20–40-minute incubation and prior to initiation of DNA synthesis, may represent some physical or chemical transformation of the potential mutation to a nonphotoreversible state through a process not connected with DNA synthesis. In this regard, the initial loss of photoreversibility appears to be correlated with initiation of RNA synthesis in the culture, although this relation may be fortuitous.

The processes responsible for loss of photoreversibility have been studied by use of Chl and DNP. While Chl specifically blocks protein synthesis, DNP blocks most of the energy-requiring reactions of the cell (including RNA, DNA and protein synthesis). DNP blocks completely the loss of photoreversibility (Table 6); therefore, energy-requiring reactions must be involved in the process. However, Chl does not block loss of photoreversibility but does block

TABLE 6

Effect of chloramphenicol and dinitrophenol on loss of mutation photoreversibility

Incubation prior to photoreversal (minutes)	Control			DNP added at 45 minutes			Chl added at 60 minutes		
	tr^+ cells per 10^6 tr^- cells		DNA (rel. amount)	tr^+ cells per 10^6 tr^- cells		DNA (rel. amount)	tr^+ cells per 10^6 tr^- cells		DNA (rel. amount)
	Dark	Light		Dark	Light		Dark	Light	
0	119.2	4.9	1.0	...	...	...	...	...	...
60	109.6	20.0	1.2	87.6	9.0	1.1	106.8	16.8	1.2
75	102.0	50.6	1.5	91.3	10.6	1.1	111.3	46.4	1.5
90	112.5	89.1	2.2	88.2	11.8	1.1	91.7	77.6	2.0
105	97.8	87.3	2.9	89.3	9.9	1.1	99.2	83.5	2.3

After UV irradiation the bacteria were incubated in liquid growth medium. To one set of three identical series of cultures, dinitrophenol (5×10^{-3} M) was added at 45-minutes incubation; to one other set chloramphenicol (20 μg per ml) was added at 60-minutes incubation. At the indicated times of incubation samples were taken, centrifuged, washed with cold minimal medium, recentrifuged and resuspended in minimal medium and held in an ice bath in the dark or exposed to intense white light for one hour (a period of time sufficient for maximum photoreversal). The increase in DNA relative to unincubated controls was determined at the times the samples were taken.

protein synthesis; it thus appears that protein synthesis is not involved in the process.

The question of involvement of protein synthesis in that earlier loss of photoreversibility which occurs prior to DNA synthesis cannot readily be tested since treatment with Chl during this period leads to some degree of "mutation frequency decline." However, DNP blocks this earlier loss of photoreversibility indicating that energy is required for the transformations involved.

These experiments suggest that the greater proportion of the potential mutations induced by UV exist in a state susceptible to photoreversal until DNA replication. Therefore, whatever the nature of the involvement of the potential mutation in the processes of mutation fixation (which occur prior to DNA synthesis), these transformations do not compromise the photoreversibility of the "potential mutation." This would seem reasonable if the potential mutation exists originally as a mutagenic nucleic acid precursor which may be "photoreversed" to a nonmutagenic condition. If this precursor is incorporated into RNA during mutation fixation as we have suggested, then it must be assumed that the mutagenic nucleotide remains photosensitive in the RNA. If, conversely, one assumes that the damage leading to mutation is induced directly in the DNA, either in one or both strands, then this DNA damage must be photoreversible until DNA replication. This question cannot be resolved until the nature of the chromophore for UV-induced mutation is established. The fact is clear, however, that the initial synthesis of DNA after UV exposure is the "copying" event leading to a photostable "error" in the genome.

DISCUSSION

Recent studies indicate that ultraviolet may induce mutation through at least two mechanisms (WITKIN and THIEL 1960; KADA, BRUN and MARXOVICH 1960). In one type of mutation induction, the initial damage is unstable and subject to modification by postirradiation treatments. It is this type of mutation induction

with which we are concerned in this paper. The second type of UV-induced mutation is stable and is not influenced significantly by postirradiation conditions. Similar considerations appear to hold for X-ray induced mutations. While a portion of the latter are influenced by postirradiation conditions, another portion is refractory to these treatments (HAAS and DOUDNEY 1958; KADA, BRUN and MARXOVICH 1960). Complete *expression* of this latter type of X-ray-induced mutation can be observed within a short period following irradiation and in the absence of DNA synthesis (KADA, DOUDNEY and HAAS 1960). RNA and protein synthesis is required for *expression* but apparently not for *induction*.

Since we do not know the chemical nature of the unstable condition which leads to UV-induced mutation at this time, we prefer to refer to it simply as the "potential mutation." While our studies suggest that the potential mutation is initially in the form of a mutagenic nucleic acid precursor formed by the action of UV on a purine or pyrimidine-containing monomer (HAAS and DOUDNEY 1957), no conclusive evidence exists to support this hypothesis. The possibility remains that the initial UV damage is to the cellular DNA or to other sites.

It is quite evident that the potential mutation can be influenced by metabolic conditions. Conditions leading to mutation require RNA and protein synthesis which is quantitatively minor and apparently not related to the gross synthesis of these macromolecules in the culture. The term "mutation stabilization" has been used to indicate those interactions increasing or supporting mutation and occurring prior to any measurable net macromolecular synthesis. The effectiveness of mutation stabilization seems to determine the frequency of mutation under ordinary conditions. In this special sense, the original view of WITKIN (1956) that protein synthesis determines the level of mutation induction is valid. While the nature of mutation stabilization is not known, it is quite attractive to interpret it as a "templating" or preparatory process to gross macromolecular synthesis in the cell. This possibility is supported by the evidence that 5-hydroxyuridine must participate in a process correlated with mutation stabilization in order to lower the induced mutation frequency (presumably through subsequent incorporation into RNA).

If mutation stabilization does not occur, it is evident that an antagonistic or competitive process occurs which removes the potential mutation from pathways leading to mutation induction. This process we have termed "mutation frequency decline." The process appears to be enzymatic since a Q_{10} of approximately two is obtained based on the rate of mutation frequency decline in the culture at various temperatures of incubation (WITKIN 1956; DOUDNEY and HAAS 1958). Energy is required since DNP blocks the process, but it is clear that protein and RNA synthesis is not required in the process. DNA synthesis is evidently not required since the process occurs in the absence of thymine as deduced from studies of UV-induced reversion of the amino acid requirement in thymine- and amino acid-requiring double auxotrophs of *E. coli* (DOUDNEY and HAAS, unpublished results).

Alternative possibilities (based on the hypothesis adopted as to the nature of the mutational chromophore) can be proposed for the mechanism leading to

mutation frequency decline. If one assumes that the potential mutation resides initially in the DNA existing at the time of UV irradiation, then the decline process may be ascribed to "repair" of the UV damage. The hypothetical repair mechanism could not involve RNA, DNA or protein synthesis, but would require energy. If the alternate view is taken that the potential mutation resides in a UV-modified nucleic acid precursor, then any process removing this precursor from the synthetic pathway of the nucleic acids involved in genetic replication would prevent mutation. In the case of the auxotrophic reversion the possibility that mutation frequency decline is due to a selective lethal effect caused by blockage of RNA or protein synthesis, on cells bearing potential mutations, cannot be eliminated although such selectivity seems extremely unlikely. In the case of the "color mutants" distinguished on EMB agar this possibility appears to be ruled out since such lethality was not observed with mutation frequency decline where the potential mutation-containing cells constituted a relatively large portion of the total survivors (DOUDNEY and HAAS 1958).

Whatever the nature of the mutation frequency decline process, it is evident that this process can be carried to completion without influencing materially the timing or the rate of subsequent macromolecular syntheses *definitely involved in mutation induction*. Treatments sufficient to reverse (or prevent) all susceptible mutations produce little or no effect on subsequent RNA, DNA or protein synthesis. For this reason, the processes involved either in mutation frequency decline or in mutation stabilization would appear to take place prior to the RNA and protein synthesis made necessary by UV irradiation to further DNA replication (HAROLD and ZIPORIN 1959; DOUDNEY 1959; DRACULIC and ERRERA 1959). It should be emphasized that these findings effectively rule out any hypothesis for mutation frequency decline based on treatments which modify only the timing of DNA synthesis so as to take advantage of decay of a "premutational" state at a constant rate (KIMBALL, GAITHER and WILSON 1959), or disappearance of "mutagens" at a rate *not influenced* by the treatment (LIEB 1960).

Postirradiation incubation in complete medium clearly leads to fixation of the potential mutation in the sense that blockage of RNA or protein synthesis will no longer promote mutation frequency decline after some synthetic event has occurred. Mutation fixation (measured by challenge procedures involving *specific* blockage of RNA or protein synthesis) appears to be correlated with initial doubling of RNA in the culture. There appears to be a relation of the amount of postirradiation RNA synthesized at the time of chloramphenicol addition to the rate of DNA synthesis in the presence of Chl and to the level of induced mutation obtained after Chl challenge. Only where the RNA and protein necessary for genetic replication is formed prior to Chl addition can DNA be produced. If this RNA and protein is not formed at the time Chl is added, the potential mutation is lost through the mutation frequency decline process. It seems quite likely that the RNA and protein involved in mutation fixation is identical with that involved in postirradiation recovery of the DNA synthesizing mechanism. It is also evident that acquisition of the *capacity* to synthesize DNA,

rather than DNA synthesis *per se*, is necessary for mutation fixation (since DNA synthesis during the Chl challenge period is unnecessary).

DNA synthesis appears to be firmly established as the terminal event in UV-induced mutation. Loss of susceptibility of the potential mutation to photoreversal appears to be intimately correlated with doubling of the DNA. Furthermore, mutation expression follows DNA synthesis and will not occur in its absence. Protein synthesis following DNA synthesis is necessary for mutation expression (HAAS and DOUDNEY 1959).

A number of hypotheses have been advanced in regard to the mechanism of mutation induction by UV. One possibility is that the UV damage occurs to the genetic DNA, and that gene action (the synthesis of RNA on the DNA template) in some manner "fixes" this damage. With subsequent DNA replication the functional mutated gene would be formed. It is difficult to explain a number of observations on this basis. The mutation frequency decline process cannot be reasonably accounted for; the 5-hydroxyuridine effect in decreasing mutation frequency remains unexplained, since it is difficult to see why RNA incorporating 5-HU would be less effective in fixing mutation according to this hypothesis than "normal" RNA. In our opinion the hypothesis for UV-induced mutation which most adequately reconciles the reported experimental data is that which we have previously advanced (DOUDNEY and HAAS 1958, 1959a). According to this hypothesis, DNA synthesis involves transfer of information from the parental DNA to the daughter DNA through an RNA-protein intermediate. On this basis, incorporation of a UV-modified RNA precursor into the RNA would lead to a "copying error" involving substitution of a different nucleotide pair in the subsequently formed daughter DNA.

Little experimental support at the molecular level exists for this indirect mechanism of DNA replication at present. A possibility which might be considered is that the DNA replicates under the usual circumstances according to the scheme proposed by WATSON and CRICK (1953), but following UV irradiation this mechanism is inactivated. In this case, the cell might revert to an alternate mechanism for DNA formation which involves an intermediate transfer of information to RNA. On this basis, we could account for most of the mutation induction data, as well as the necessity for RNA and protein synthesis in DNA replication following UV irradiation.

It is quite clear that none of the above hypotheses or speculations have been established and that a considerable amount of further experimentation is necessary before mutation induction will be understood. However, the investigations reported here indicate some of the directions which future experimentation must take. Moreover, in view of the mesmeric effect of the WATSON and CRICK hypothesis for DNA replication on research in this area, with its support at the enzymatic level by the definitive studies of KORNBERG and co-workers, it should be pointed out that no conclusive evidence has been reported to support this direct complementary scheme for DNA replication, either in *living systems* or for formation of *functioning* genetic DNA. Accordingly, other less direct mech-

anisms for DNA replication must be thoroughly investigated and considered. This is especially true in understanding UV-induced genetic change which clearly involves not only DNA synthesis, but also synthesis of the other primary information-bearing macromolecules of the cell, RNA and protein.

SUMMARY

Postirradiation treatments blocking RNA or protein synthesis lower markedly the ultraviolet-induced mutation frequency (reversion of tryptophan requirement) in *Escherichia coli* strain WP2. Treatments of less than 20 minutes reduce the mutation frequency to a minimal level without significant modification of the time or rate of subsequent synthesis of RNA, DNA and protein. Therefore, the mutation frequency decline process apparently is an active process, probably enzymatically mediated, and not dependent on delay in postirradiation macromolecular synthesis. The progressive loss of susceptibility of the potential mutation to these treatments is closely correlated with the progression of ribonucleic acid synthesis in the culture. A relation exists between the amount of RNA synthesized at the time of chloramphenicol addition, the relative rate of DNA synthesis in the presence of chloramphenicol, and the induced mutation frequency. Apparently, only when the RNA and protein necessary for genetic replication is synthesized prior to chloramphenicol addition will DNA be formed. When the RNA and protein involved has not been formed, the potentiality for mutation is lost through the active decline process.

Decline in induced mutation frequency is promoted by 5-hydroxyuridine after a considerable lag. This decline is correlated with the doubling of RNA in the culture suggesting that the analogue causes the decline through incorporation into the RNA. However, loss of *susceptibility* to the 5-hydroxyuridine-promoted reduction in mutation frequency occurs prior to measurable RNA synthesis and is correlated with the *mutation stabilization* process. Incubation with 5-hydroxyuridine of sufficient duration to prevent all sensitive mutations does not delay DNA synthesis.

Several lines of evidence indicate that DNA synthesis is the terminal event in UV-induced mutation. However, delay of DNA synthesis does not modify UV-induced mutation frequency in the absence of the active decline process unless drastic treatments are employed which grossly alter cellular metabolism. Loss of mutation photoreversibility is correlated with DNA synthesis in the culture. Dinitrophenol (but not chloramphenicol) blocks the loss of mutation photoreversibility.

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POSTNATAL CHANGES IN THE INTENSITY OF COAT COLOR IN DIVERSE GENOTYPES OF THE GUINEA PIG[1,2]

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THE fur of guinea pigs is well developed at birth. As the pigment has been produced under almost constant conditions, this initial intensity has seemed better adapted for intensive analysis of the interaction effects of genes than that after exposure of the skin to the effects of varying temperatures and nutritive conditions. Such an analysis has been the subject of a number of preceding papers (WRIGHT 1949, 1959a,b,c, 1960a,b).

Visual grades were, however, also assigned to adults in the colony at the times of birth of their litters in the same way as to their young, by comparison with standard squares of fur, numbered in such a way that each grade was barely distinguishable from the adjacent ones (eumelanics ranging from 1, near-white, to 21 for intense black; phaeomelanics from 1, also near-white, to 13, the most intense yellow or "red"). Indices derived from reflectionmeter readings (cf. WRIGHT 1959c) were also obtained in 1952–1954 for parents as well as young. The average index for intense black was 41.3, for intense brown 34.8, and for intense yellow 22.7 while that for white was zero by definition.

As the amount of pigment per unit weight of hair had been determined colorimetrically in many animals to which visual grades had also been given (RUSSELL 1939; HEIDENTHAL 1940; WRIGHT and BRADDOCK 1949), it was possible to derive formulae by which the amount of pigment could be estimated either from visual grades or reflectionmeter indices (WRIGHT 1949, 1959c).

It has been convenient to recognize seven major series of self colors, ranging from relatively intense (with *C* present) through grades of dilution (with lower *c* compounds) to pure white ($c^a c^a$, $eec^r c^{ra}$ or $Ec^{kd}c^{kdra}ppff$ in which $c^r c^{ra}$ includes $c^r c^r$ and $c^r c^a$ and similarly with $c^{kd}c^{kdra}$).

Black, dark-eyed dark sepia	*EaaBP-*
Pink-eyed pale sepia	*EaaBppF*
Brown-eyed dark brown	*EaabbP-*
Pink-eyed pale brown	*EaabbppF*
Pink-eyed pale brownish cream	*Eaa-ppff*
Stable yellow	*ee---F*
Fading yellow	*ee---ff*

[1] Paper No. 800 from the Department of Genetics, University of Wisconsin.

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The same eumelanic color as found with *E* and the same phaeomelanic color as found with *ee* are combined in a mosaic pattern in tortoise-shells with $e^p e^p$ or $e^p e$, and in a banding pattern in each hair in agoutis with *EA*. The visual grades refer to the prevailing color on the backs of self-colored animals (*Eaa* or *ee*) or the unmixed areas on the backs of tortoise-shells. The reflectionmeter readings were taken at the darkest point near the middle of the back.

Second order variations in intensity of all series have been due to genes *Dm*, *dm*; *Si*, *si* (WRIGHT 1959a,b,c); and in the case of pale eumelanics also to less well-identified modifiers of which the leading pair is represented by Mp_1, mp_1 (WRIGHT 1960a). The pairs *Dm*, *dm*; *Si*, *si* have been studied most thoroughly in the dark sepias and stable yellows and especially in compounds involving lower *c* compounds, $c^d c^d$, $c^d c^r$, $c^d c^a$, $c^r c^a$, in which the dilution effects of *dm* and *si* are most pronounced. As the effects of these modifiers on intensity are rather similar, it is convenient to group genotypes according to the number of plus modifiers (4 = *DmDmSiSi*, 3 = *DmdmSiSi* and *DmDmSisi*, 2 = *dmdmSiSi*, *DmdmSisi* and *DmDmsisi*, 1 = *dmdmSisi* and *Dmdmsisi*). Genotypes with *dmdmsisi* are pure white except for occasional very pale spots on the head. Browns of genotype *EbbCP* are subject to dilution of a peculiar sort (known as dinginess) in which genes *C*, *P* and *F* reduce color above the optimum *CPpff* (WRIGHT 1947, 1960a).

Postnatal changes: Tables 1 to 6 give the indices (I_1). These have been transformed into the relative amounts of pigment (M_1) at the time of the first litter after reflectionmeter readings began to be taken. Actual first litters are usually born when the parents are about one half year old, but many of the matings on hand when reflectionmeter readings began to be taken had already had one or more litters. The most important changes in intensity have, however, occurred by one half year of age in most colors. The standard of comparison (100) is intense black (*EBCP*) for dark and pale sepias, intense brown (*EbbCPp*) for dark and pale browns, and intense yellow (*eeCFF*) for all yellows. Pale brownish creams (*ECppff*) have been compared both with browns and yellows. The columns headed 100 M_1/M_o give the percentages that the estimates at birth of first litter form of the estimates for the same genotypes (many more individuals) at birth. These percentages are averaged for each *c* compound (or group of compounds in some cases) in the last columns.

It may be seen that there are marked changes in intensity in many cases and that these are not all in the same direction even in the same series of colors.

More critical though less extensive evidence of change can be obtained by restricting consideration to cases in which the same individuals were graded at birth and at their first litter. This is done in Tables 7 to 11 which give the index, I_1, the change from birth (Δ_{1-0}) and value of t from paired comparisons. The average index at birth of litters after the first (I_2), the difference from that at birth of the first litter (Δ_{2-1}) and the value of t from these paired comparisons are also given. It should be noted that these comparisons of later changes are not based wholly on the same animals as those on which the initial changes are

based. Significance at the .05, .01 and .001 levels is indicated by one, two or three asterisks, respectively.

These tests support the more important changes indicated in Tables 1 to 6. The greatest decrease in intensity of yellow after birth is in *Cff* ("fading" yellow), but there are highly significant decreases in "reds" (*CFF*, *CFf*). The term stable "yellow" is, in fact, somewhat of a misnomer. Those with c^kc^k decrease slightly but significantly, and those with c^kc^{ra} decrease greatly. On the other hand, those with c^dc^d and three or four plus modifiers increase slightly but significantly, and those with c^dc^{ra} increase considerably more. Those with c^kc^d

TABLE 1

Indices (I_1), *percentages which estimates of amount of melanin* (M_1) *form of the more numerous estimates at birth* M_0 *of yellows, graded at the time of first litter* (*usually 5 or 6 months*)

Yellow genotype	No.	At first litter		Av.
		I_1	$100\ M_1/M_0$	
eeCFF except *sisi*	22	18.5	63.3	65.5
sisi	3	18.3	76.6	
Ff	8	17.1	67.5	
c^kc^kFF (4,3)	20	12.8	83.4	84.7
(2)	1	12.3	107.1	
Ff	2	10.2	86.3	
c^kc^dFF (4)	4	16.6	101.2	109.5
(2)	1	15.7	142.5	
$c^kc^{ra}FF$ (4)	4	8.1	73.3	70.1
(3)	17	6.8	66.3	
(2)	6	6.1	78.6	
c^dc^dFF (4)	9	18.4	126.1	109.0
(3)	13	15.5	116.0	
(2)	30	12.2	100.0	
(1)	15	11.1	109.2	
Ff	2	11.5	119.2	
$c^dc^{ra}FF$ (4)	5	13.6	146.9	122.5
(3)	11	11.8	152.3	
(2)	18	7.7	126.1	
(1)	27	3.3	103.5	
C ff	19	8.6	50.8	50.8
c^dc^dff	1	1.1	39.4	39.4
ECppff	32	2.5	44.3	44.3

In most cases the number of plus modifiers (*Dm,Si*) is indicated in parentheses. *ECppff* (pale brownish cream) is included as if yellow.

TABLE 2

Data on dark sepias, graded at the time of first litter, similar to those for yellows in Table 1

Dark sepia genotype	No.	At first litter I_1	At first litter $100\,M_1/M_0$	Av.
ECP	17	40.2	89.7	89.7
c^kc^{kdr} (4,3)	18	39.3	83.7	85.1
(2)	2	39.2	98.0	
c^kc^a (4,3)	6	38.4	94.2	99.8
(2)	1	37.6	116.5	
(1)	1	34.0	117.1	
c^dc^d (4)	3	37.3	93.2	122.2
(3)	3	37.5	110.7	
(2)	12	37.2	125.3	
(1)	3	36.2	150.2	
c^dc^r (4,3)	2	39.1	102.0	114.1
(1)	2	38.2	126.3	
c^dc^a (4)	5	36.5	131.0	151.8
(3)	4	34.1	125.1	
(2)	9	33.6	155.1	
(1)	17	29.3	162.5	
c^rc^r (4,3)	10	41.1	106.1	106.1
c^rc^a (4,3)	19	38.2	160.6	162.8
(2)	7	35.4	157.2	
(1)	7	32.4	174.4	

TABLE 3

Data on dark browns, excluding dingy browns, graded at the time of first litter, similar to those for yellows in Table 1

Dark brown genotype	No.	At first litter I_1	At first litter $100\,M_1/M_0$	Av.
CPF non *dn*	32	31.2	78.0	...
c^kc^kPF	3	29.8	72.2	82.3
c^kc^aPF	1	30.8	112.5	
c^dc^d (4,3)	5	29.7	83.5	82.4
(2)	2	26.5	77.4	
c^dc^r (2)	1	32.2	86.9	
c^dc^a (2,1)	4	29.2	146.8	...
c^rc^r	6	33.0	90.5	...

TABLE 4

Data on dark browns carrying C, *at time of first litter, similar to those for yellows in Table 1 except that sires and dams are dealt with separately*

Dark brown genotype	Dinginess	Sire at first litter			Dam at first litter		
		No.	I_1	$100\ M_1/M_0$	No.	I_1	$100\ M_1/M_0$
ECPF	non *dn*	6	31.3	78.6	6	32.9	87.6
ECPff	non *dn*	7	31.4	75.5	7	31.2	74.5
e^pe^pCPF	non *dn*	1	28.4	69.5	5	29.6	75.6
Total	non *dn*	14		76.4	18		79.2
ECCPPFF	*dn*+ (SH)	16	23.1	78.6	17	29.1	122.3
ECCPPFF	*dn*— (SH)	11	17.1	86.0	9	21.4	124.3
$e^pe^pCCPPFF$	*dn*+ (SH)	2	18.5	68.0	3	20.1	78.0
e^pe^pCPPFf	*dn*+ (SF)	2	21.2	64.7	2	31.9	142.0
e^pe^pCPPff	*dn*+ (SF)	8	24.8	64.6	9	28.5	84.6
Total	*dn*	39		76.6	40		111.9

The estimates at birth (M_0) with which the estimates at first litter are compared, include both males and females as there is no significant difference at this time. The symbol *dn* refers to dinginess; *dn*+ and *dn*— refer to the darkest and lightest dorsal areas on dingy browns. The symbols in parentheses are strain designations.

TABLE 5

Data on pale sepias, graded at time of first litter, similar to those for yellows in Table 1

Pale sepia genotype	No.	At first litter		Av.
		I_1	$100\ M_1/M_0$	
BCppFF	24	13.6	42.8	47.5
Ff	8	15.1	61.4	
c^kc^kFF	5	14.4	68.1	62.4
c^kc^rFF	6	9.2	57.7	
c^dc^dFF (4)	3	5.9	26.4	46.6
(3)	2	7.9	50.2	
(2)	3	9.0	61.3	
(1)	2	6.7	51.0	
c^dc^rFF	2	7.9	...	...
c^dc^aFF (4)	3	7.2	78.8	63.8
(3)	3	5.8	67.5	
(2)	8	5.1	58.1	
(1)	4	4.2	61.2	
c^rc^rFF	23	7.5	96.8	96.8
c^rc^aFF	1	2.8	100.0	(100.0)

TABLE 6

Data on pale browns, graded at time of first litter, similar to those for yellows in Table 1. Pale brownish creams (ECppff) *are entered here as well as in Table 1*

Pale brown genotype	No.	At first litter		Av.
		I_1	$100\ M_1/M_0$	
bbCppFF	10	15.4	67.1	65.8
Ff	13	13.7	64.8	
c^kc^kFF	1	15.3	90.6	90.6
c^dc^dFF	4	13.0	68.9	68.9
c^dc^aFF (4)	6	6.8	44.9	58.9
(3)	3	9.3	87.0	
c^rc^rFF	5	5.1	81.7	81.7
ECppff	32	2.5	46.7	46.7

TABLE 7

The number of yellows of various genotypes with indices at both their own birth and at their first litter I_1*; the amount of change* (Δ_{1-0}) *and the value of t from the paired comparisons; the number with more than one index after birth, the average for all later indices* (I_2), *the change from* I_1 *to* I_2 (Δ_{2-1}) *and the value of t*

Yellow genotype	First adult grade				Av. later grade			
	No.	I_1	Δ_{1-0}	t	No.	I_2	Δ_{2-1}	t
eeCFF	7	18.0	−3.3	4.5**	13	18.3	−0.2	0.7
Ff	5	16.6	−4.8	7.8**	2	15.8	−0.4	1.3
c^kc^kFF	11	12.9	−0.8	2.5*	10	13.6	+0.8	2.7*
c^kc^dFF	3	16.6	+1.0	0.7	2	14.4	−1.6	1.1
c^kc^raFF (4,3)	6	7.5	−2.0	4.6**	9	7.3	+0.4	1.1
(2)	5	6.2	−1.1	2.0	3	7.1	+0.4	0.6
c^dc^dFF (4,3)	17	16.1	+1.1	3.8**	8	17.2	−0.5	1.5
(2,1)	34	11.9	+0.0	0.2	28	12.2	+0.1	0.8
c^dc^raFF (4,3)	10	12.5	+2.4	4.2**	9	13.4	+0.5	1.7
(2,1)	21	5.4	+0.8	2.3*	26	4.8	+0.0	0.1
Cff	7	7.8	−5.3	9.6***	7	8.9	−0.5	0.9

* Significance at the .05 level.
** Significance at the .01 level.
*** Significance at the .001 level.

TABLE 8

Data on changes in intensity of dark sepias (EBPF), *similar to those for yellows in Table 7*

Dark sepia genotype	First adult grade				Av. later grade			
	No.	I_1	Δ_{1-0}	t	No.	I_2	Δ_{2-1}	t
CF	7	40.2	−0.4	1.2	9	40.8	+0.4	1.1
c^kc^k	7	39.7	−1.2	2.1	5	40.8	+0.9	1.7
c^kc^r	8	39.5	+0.7	1.5	7	41.1	+1.3	1.9
c^kc^a	3	37.9	−0.4	2.3	5	39.3	+1.0	4.5*
c^dc^d (4,3)	5	37.2	−0.2	0.5	1	40.3	+1.9	..
(2,1)	13	36.9	+2.7	4.6***	7	37.3	+0.1	0.2
c^dc^r (2)	2	38.2	+2.1	0.5	1	35.2	0.0	..
c^dc^a (4,3)	5	34.5	+1.7	1.5	3	38.5	+2.1	3.9
(2)	6	33.9	+7.2	10.3***	8	34.0	+0.5	0.8
(1)	7	30.6	+7.9	7.5***	8	31.7	+1.5	1.5
c^rc^r (4,3)	5	41.1	+0.7	0.4	7	40.9	−0.5	1.5
c^rc^a (4,3)	12	38.7	+5.0	12.9***	11	38.9	+1.2	1.9
(2)	7	35.4	+6.8	7.2***	2	38.1	+0.9	..
(1)	7	32.4	+8.0	5.5**	5	36.2	+3.6	2.3

See footnotes Table 7.

TABLE 9

Data on changes in intensity of dark browns (EbbPFF), *similar to those for yellows in Table 7*

Dark brown genotype	First adult grade				Av. later grade			
	No.	I_2	Δ_{1-0}	t	No.	I_2	Δ_{2-1}	t
C non *dn* male	9	31.1	−4.4	5.1***	8	30.7	−0.8	2.1
female	11	32.1	−3.1	3.0*	6	31.2	+0.4	0.4
dn+(SH) male	5	22.6	+0.6	0.2	7	23.4	+0.3	0.2
female	7	31.0	+3.4	2.7*	9	29.8	+3.2	3.1*
dn−(SH) male	5	18.4	+1.4	0.7	4	14.1	−2.2	0.7
female	4	21.0	+2.5	0.8	3	23.2	+2.9	1.1
C non *dn* (tot.)	20	31.6	−3.7	5.4***	14	30.9	−0.3	0.6
c^kc^k	2	29.1	−5.6	..	2	31.8	+0.8	..
c^dc^{dr}	6	28.9	−3.2	10.0***	4	30.1	+1.2	3.6*
c^dc^a	4	29.2	+2.4	3.2*	4	30.0	+0.8	0.8
c^rc^r	4	33.5	−0.1	0.3	4	34.6	+1.8	1.1

See footnotes Table 7.

TABLE 10

Data on changes in intensity of pale sepias (EBppF), *similar to those for yellow in Table 7.* FF *is present in all lower* c *compounds*

Pale sepia genotype	First adult grade				Av. later grade			
	No.	I_1	Δ_{1-0}	t	No.	I_2	Δ_{2-1}	t
CFF	9	15.1	—7.9	5.0***	18	12.1	—1.4	3.5**
CFf	6	15.4	—6.5	6.4**	8	10.3	—4.7	5.1**
c^kc^k	5	14.4	—5.5	9.8***	4	10.8	—2.9	3.5*
c^kc^k or c^kc^r	5	15.8	—5.9	9.4***	1	9.8	—7.5	..
c^kc^r	5	8.7	—4.1	7.8**	4	7.9	—1.2	4.9*
c^dc^d	6	8.3	—3.7	2.7*	9	5.7	—1.8	3.6**
c^dc^a (4,3)	5	7.4	—3.7	3.1*	6	4.8	—1.7	3.3*
c^dc^a (2,1)	7	4.5	—2.8	7.9***	10	3.8	—1.0	2.6*
c^rc^r	3	6.8	—0.6	0.3	8	8.9	+0.2	0.5

See footnotes Table 7.

TABLE 11

Data on changes in intensity of pale browns (EbbppF) *and pale brownish creams* (Eppff), *similar to those for yellow in Table 7.* FF *is present in all lower* c *compounds*

Pale brown genotype	First adult grade				Av. later grade			
	No.	I_1	Δ_{1-0}	t	No.	I_2	Δ_{2-1}	t
CFF	3	14.8	—3.7	2.1	7	13.9	—1.1	1.8
CFf	5	15.8	—1.8	2.9*	8	14.1	—0.9	1.5
c^kc^k	1	15.3	—1.3	..	2	10.6	—0.2	..
c^dc^d	4	13.0	—3.6	2.6	2	11.2	—2.2	..
c^dc^a	5	9.1	—4.7	13.3***	3	8.7	+0.5	0.7
Cff	18	2.8	—2.3	6.4***	20	2.2	—0.5	2.5*

See footnotes Table 7.

seem to behave more like c^dc^d than c^kc^k, but the numbers are too small for any confident assertion. There are no important changes following the first litter.

In the case of the dark sepias, the decreases in intensity of *C* and c^kc^{kdr} in Table 2 are not supported to the extent of significance in Table 8, but the increases in intensity of c^rc^a (with any number of plus modifiers) and of c^dc^d and c^dc^a with one or two plus modifiers are shown to be highly significant. It is remarkable that the c^d dark sepias with different numbers of modifiers tend to converge after birth while the c^dc^{ra} yellows tend to diverge.

There is only one later change in Table 8 that is significant at the .05 level, and it is so small in absolute amount that it may reflect merely an accidentally small variance of differences. There is, however, a prevailing slight increase in later litters that must be given some weight. This did not hold for yellows.

Among the dark browns (Table 9) there are highly significant decreases in intensity of nondingy intense (*C* non *dn*) and of c^dc^{dr} but a probably significant increase in the case of c^dc^a. The only significant change in later litters is an

increase in c^dc^{dr} which partly compensates for the initial decrease and an increase in dingy intense females that extends a significant increase in these after birth.

More extensive data on *C* browns are given in Table 4. Both males and females that are nondingy (largely *Pp*) become much lighter after birth (21 to 24 percent), but in all categories of dingies the females either fade less than the males or become more intense (males lighten about 23 percent while females darken about 12 percent on the average). These agree with earlier results (WRIGHT 1947) and with the behavior of dingy brown modified by a gene "whitish" (*W*) described by IBSEN (1932) and IBSEN and GOERTZEN (1951).

Table 5 shows a marked lightening of all pale sepia genotypes after birth except in the case of c^rc^r and c^rc^a. The tests of significance of individual changes in Table 10 show that these decreases are significant and that the lightening continues to later ages in this case.

The pale browns also decrease in intensity (Table 6). The numbers of individuals graded at birth and first litter were very small (Table 11), but significant decreases are shown in two cases (*CFf* and c^dc^a). There is probably continued lightening, but none of the differences reach significance. From comparison of Tables 5 and 6 it appears that the *C* pale browns do not lighten as much as the *C* pale sepias, but more data are needed.

The pale brownish creams (*Eppff*) are included in both Tables 1 and 6. The color in an earlier period was indistinguishable from pale yellow but had shifted halfway to pale brown from a shift in modifiers by 1952 (WRIGHT 1960b). The drastic decrease in intensity (to 47 percent of the intensity at birth if treated as eumelanic (Table 6) in comparison with 44 percent treated as phaeomelanic (Table 1)) is clearly significant (Table 11).

Comparisons with earlier results: The estimates of amount of pigment from the reflectionmeter readings at birth and later are compared with the estimates made previously from visual grades in Tables 12 to 16. There is agreement in the main features but there are differences that are obviously too great to be due to sampling errors. There are several possible reasons. There is some danger, first, of subjective error in the case of the visual grades, and second, of error from shifting standards (since the standard fur samples tended to fade slowly and required restandardization from time to time). It is unlikely that the average grades at birth are affected appreciably by these causes. Third, there was more uncertainty in the visual grades of the adults whose coats differed markedly from the standard samples because of longer hair and different texture. Some of the comparisons suggest conservatism in recording changes from the grades at birth. This does not hold in all cases, however, and is probably only a minor factor if it held at all. In the fourth place, it must be noted that the two sets of estimates were not estimates of quite the same thing. In the visual grades, the fur in the general middorsal region was compared with the standard fur samples. A reflectionmeter reading, on the other hand, applies only to a very small area. This was, perhaps unfortunately, taken at the most intense region along the middorsal line. The ratio of the intensity of this in a given genotype to that in intense black (as 100) or intense yellow (as 100) need not be the same as that in a more extensive area.

TABLE 12

Comparison of amounts of pigment in yellows estimated from visual grades at birth and as adults (1949) and from reflectionmeter readings at birth and at birth of first litter (1960). In the former case the number of plus modifiers was not known, in the latter the averages apply to those with three or four plus modifiers

	Pigment at birth				Pigment in adults			
	1949		1960		1949		1960	
Genotype	No.	M_0	No.	M_0	No.	M_1	No.	M_1
CFF	871	100.0	65	100.0	85	76.1	22	63.3
c^kc^k	315	36.1	33	37.9	51	30.0	20	31.6
c^kc^d	58	38.6	32	44.7	3	40.2	4	50.8
c^kc^r	301	17.9	75	19.3	49	12.1	21	12.9
c^kc^a	353	17.1	..	...	27	12.0	.	...
c^dc^d	404	35.7	132	41.5	35	36.9	22	51.4
c^dc^r	405	13.6	86	19.9	46	16.0	16	30.0
c^dc^a	436	14.0	..	...	41	16.5	..	...
c^rc^r	Many	0.0	..	0	Many	0.0	..	0.0
c^rc^a	Many	0.0	..	0	Many	0.0	..	0.0
c^ac^a	Many	0.0	..	0	Many	0.0	..	0.0
CFf	454	85.3	39	79.8	27	52.3	8	53.9
Cff	697	34.5	55	33.3	87	10.8	19	16.9
c^kc^kff	106	4.5	3	3.5	25	0.1	..	...
c^dc^dff	495	5.1	10	3.8	74	0.6	1	1.5
ECppff	113	6.2	122	8.2	29	3.0	32	3.6

TABLE 13

Comparisons of estimated amounts of pigment in dark sepias analogous to those for yellows in Table 12

	At birth				Adult			
	1949		1960		1949		1960	
Genotype	No.	M_0	No.	M_0	No.	M_1	No.	M_1
CFF	780	100.0	66	100.0	105	95.6	17	89.7
c^kc^k	48	88.6	..	...	8	80.5	..	...
c^kc^d	12	81.6	71	98.1	.	...	18	82.1
c^kc^r	46	93.5	..	...	5	92.3	..	...
c^kc^a	206	74.7	46	79.6	25	72.8	6	75.0
c^dc^d	151	66.6	20	64.9	25	75.8	6	67.8
c^dc^r	150	72.4	20	78.8	27	79.4	2	80.4
c^dc^a	457	38.7	57	42.1	91	54.5	9	55.8
c^rc^r	172	81.2	49	92.4	30	86.1	10	98.1
c^rc^a	324	42.4	68	45.8	56	70.8	19	73.5
c^ac^a	Many	0.0	..	0.0	Many	0+	..	0+

TABLE 14

Comparisons of estimated amounts of pigment in dark browns (EbbP) *analogous to those for yellows in Table 12*

	At birth				Adult			
	1949		1960		1949		1960	
Genotype	No.	M_0	No.	M_0	No.	M_1	No.	M_1
C	1,962	100.0	198	100.0	271	73.5	32	78.0
$c^k c^k$	202	96.1	..	...	17	84.0	3	70.8
$c^k c^d$	31	101.6	8	98.6	...	...	..	...
$c^k c^r$	90	97.4	..	...	6	76.3	..	...
$c^k c^a$	232	83.0	6	67.9	24	70.0	1	75.9
$c^d c^d$	82	84.6	7	84.8	.	..	5	70.4
$c^d c^r$	41	95.5	4	96.4	1	70.0	..	...
$c^d c^a$	143	63.2	23	67.1	26	69.3	..	...
$c^r c^r$	72	95.0	27	98.0	15	77.0	6	88.2
$c^r c^a$	224	63.9	11	70.9	39	70.9	..	...
$c^a c^a$	Many	0.0	..	0.0	Many	0+	..	..

TABLE 15

Comparisons of estimated amounts of pigment in pale sepias (EBppF) *analogous to those for yellows in Table 12. Standard 100 for intense black*

	At birth				Adult			
	1949		1960		1949		1960	
Genotype	No.	M_0	No.	M_0	No.	M_1	No.	M_1
CFF	468	17.1	60	22.1	101	7.5	24	8.9
$c^k c^k$	99	15.1	26	14.2	20	4.1	5	9.6
$c^k c^d$	21	10.9	4	15.9	1	7.7	..	...
$c^k c^r$	39	9.1	13	9.0	7	5.1	6	5.2
$c^k c^a$	147	6.2	11	8.8	28	2.6	..	...
$c^d c^d$	168	5.3	13	9.8	16	2.8	5	3.5
$c^d c^r$	158	3.3	..	...	30	2.0	2	4.3
$c^d c^a$	172	2.4	35	4.6	52	1.3	6	3.4
$c^r c^r$	202	2.0	41	3.1	21	2.9	23	4.0
$c^r c^a$	92	0.5	17	1.3	24	0.6	1	1.3
$c^a c^a$	Many	0.0	..	0.0	Many	0.0	.	0.0
CFf	108	11.4	67	16.9	35	4.0	8	10.4

TABLE 16

Comparisons of estimated amounts of pigment in pale browns (EbbppF) *analogous to those for yellows in Table 12. Standard 100 for intense brown*

	At birth				Adult			
	1949		1960		1949		1960	
Genotype	No.	M_0	No.	M_0	No.	M_1	No.	M_1
CFF	209	27.6	38	33.5	39	18.9	10	22.5
c^kc^k	244	22.6	1	24.6	1	9.6	1	22.3
c^kc^d	10	16.7	..	...	..	...	..	...
c^kc^r	100	16.3	.	...	1	21.8	..	...
c^kc^a	50	13.1	1	17.6	8	4.7	..	...
c^dc^d	7	14.2	23	25.5		...	4	17.6
c^dc^r	3	7.3	10	16.1	2	1.0	..	...
c^dc^a	23	4.0	28	15.6	3	2.1	9	8.8
c^rc^r	35	4.1	23	6.6	3	5.3	5	5.4
c^rc^a	60	0.9	4	2.9	13	0.9	..	...
c^ac^a	Many	0.0	..	0.0	Many	0.0	..	0.0
CFf	20	22.3	67	29.2	8	12.3	13	18.9

This holds especially of sepias. A new born black with gene *C* is of a uniformly saturated black all over its back, while the intensity of lower *c* compounds falls off laterally (as does a *C* red). The striking differences between the values for dark sepias of genotypes c^kc^{kdr} and c^rc^r in the two sets of estimates (Table 13) is undoubtedly due to this cause. The midline of these was usually as intense a black as in *C* blacks, but the color often showed a slightly brown tinge laterally. These near-blacks were in fact not graded by comparison with a black fur sample (which soon acquires a slight brownish tinge) but with a recently born *C* black with no tinge of brown (even on the belly) as the standard for grade 21. An animal that showed any brownish tinge laterally was assigned grade 20 even though as black as possible along the midline. There are probably small differences due to this cause in other cases. Finally, the most important cause of differences was undoubtedly difference in the prevailing array of modifiers. The modifiers *Dm*, *dm*; *Si*, *si* were not understood until after all of the records discussed in 1949 had been made. Extreme dilutes (largely those with only one plus modifier) were indeed not mated in the series (closed in 1944) from which the 1949 records were taken, and there were very few of these among the young that were graded. Many of the young, however, undoubtedly had only two plus modifiers though three or four must have been more common. The 1960 averages (from animals born in 1952–1954) are restricted to those with three or four modifiers (Tables 12 to 16). This restriction is probably largely responsible for such differences as the much greater intensity of the c^d yellows in 1960 as compared with 1949 since *dm* had been especially abundant in these. The estimates for the pale sepias and pale browns are more erratic than in the other cases. This is due in part to small numbers and in part to segregation of modifiers of the type of *Mp*. There is also an obvious systematic difference, the 1960 estimate being

much higher in most cases but especially where c^d was involved. At least two minus modifiers (mp_1, mp_2) had been very common in the colony at an early period and caused so much difficulty in distinguishing *c* compounds that pale sepias and pale browns that were considered abnormally pale for their genotypes were systematically rejected in making matings (WRIGHT 1960a). The average grades, thus, were much higher in 1952–1954, when the reflectionmeter readings were taken, than they had been up to 1944, the last year on which the 1949 averages are based.

In view of these differences, it is obvious that no simple meaning can be attributed to any sort of average of the 1949 and 1960 estimates. Each should be considered separately for what it is (for the fullest understanding of the age effects). Nevertheless, the discussion of these effects is facilitated so much by reduction to single sets of figures for pigmentation at birth and in adults that we present the unweighted averages of the two sets in Table 17 and Figures 1 and 2. Where a genotype was represented in only one set, the average is given in parentheses, and modified in the case of pale sepias and pale browns as described in the caption. Amounts of dark and pale brown are given here relative to 50 for intense brown which puts them on approximately the same basis as the sepias and thus relative to 100 for intense black.

Scheme of interactions: It is obvious that the differences in direction and amount of change cannot be accounted for by any single process or pair of opposed processes. This is not surprising in view of the number of loci that are concerned and consequent minimum number of processes at which rate may change with changes in age or environmental conditions. Figure 3 is a variant of

TABLE 17

Unweighted averages of estimates of amounts of pigment from visual grades (1949) and from reflectionmeter readings (1960) at birth (M_0) *and as adults* (M_1)

	EBP		*EBppF*		*EbbP*		*EbbppF*		*Eppff*		*eeFF*		*eeff*	
Genotype	M_0	M_1	M_0	M_1	M_0	M_1	M_0	M_1	M_0	M_1	M_0	M_1	M_0	M_1
C	100	93	20	8	50	38	15	10	7	3	100	70	34	14
c^kc^k	93	81	15	7	49	39	12	8	0	0	37	31	4	(0+)
c^kc^d	90	(82)	13	..	50	..	(12)	..	0	..	42	45	(6)	(0+)
c^kc^r	96	87	9	5	49	(38)	(12)	..	..	..	18	13	..	..
c^kc^a	77	74	8	(4)	38	37	8	(4)	0	..	18	13	(0+)	(0+)
c^dc^d	66	72	8	3	42	(35)	10	(6)	0	0	39	44	4	1
c^dc^r	76	80	(5)	3	48	..	6	(2)	..	..	17	23	(0+)	(0)
c^dc^a	40	55	4	2	33	(35)	5	3	0	..	17	23	(0+)	(0)
c^rc^r	87	92	3	4	48	41	3	3	0	..	0	0	(0)	(0)
c^rc^a	44	72	1	1	34	(35)	1	(1)	0	..	0	0	(0)	(0)
c^ac^a	0	0+	0	0	0	0+	0	0	0	0	0	0	(0)	(0)
CFf	100	93	14	7	50	38	13	8	..	..	83	53	..	..

Where only one estimate was available this is put in parenthesis. In the case of pale sepia (*EBppF*) and pale browns (*EbbppF*) the marked systematic difference is partially allowed by multiplying by 1.5 if the single estimate is for 1949 and by 0.67 if for 1960. Cases in which only one individual was available are omitted. Standard for brown and pale brown is 50 for *EbbCP*.

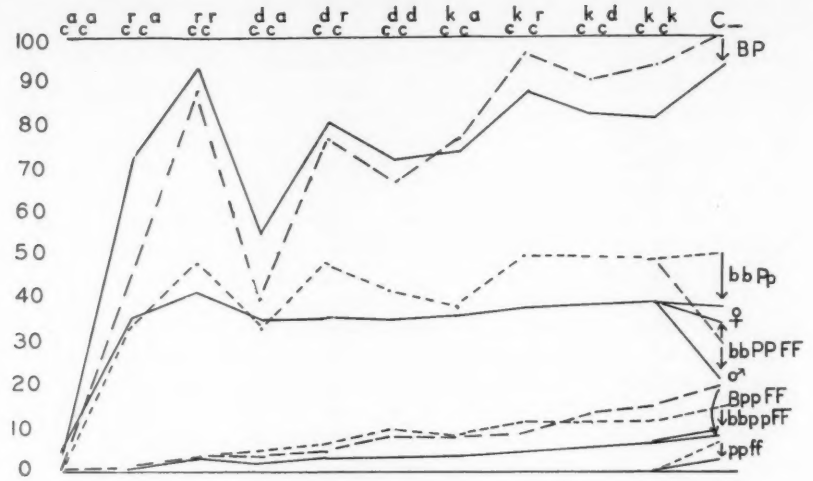

FIGURE 1.—Estimates of amounts of pigment in eumelanic genotypes at birth (broken for sepias, dotted for browns) and as adults from Table 17.

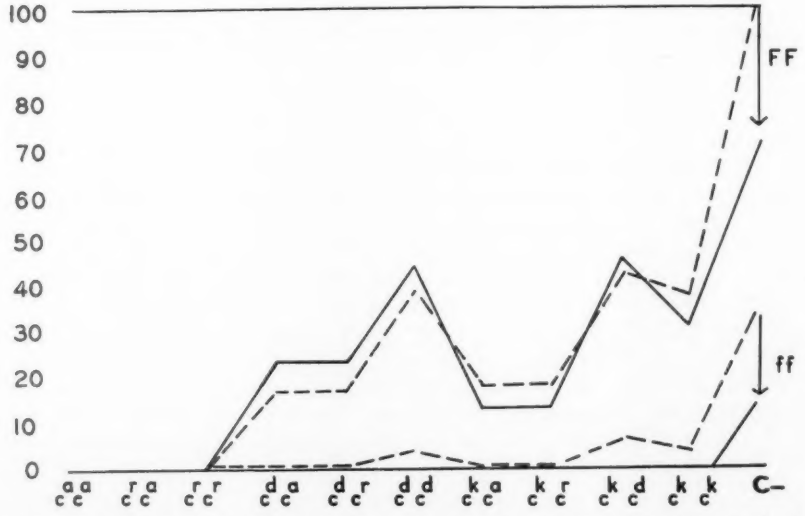

FIGURE 2.—Estimates of amounts of pigment in phaeomelanic genotypes at birth (broken) and as adults from Table 17.

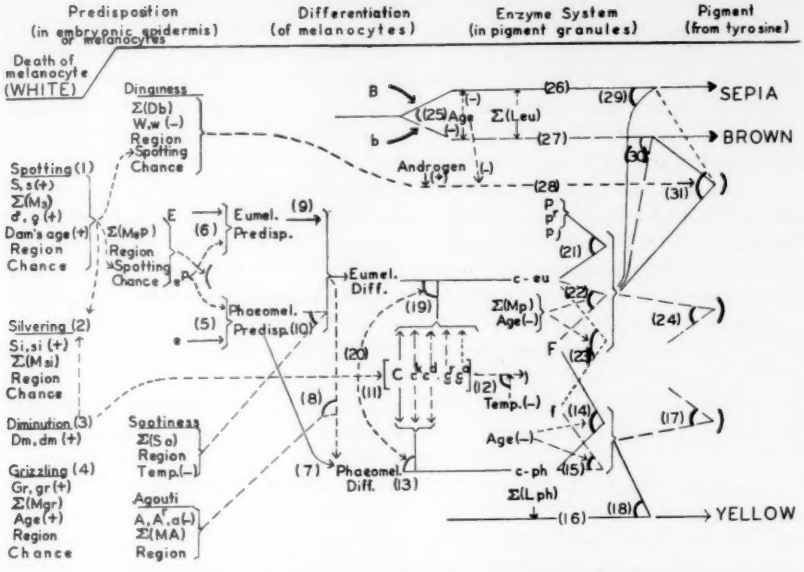

FIGURE 3.—A hypothetical scheme of sequences of reactions controlled by known genes, residual genetic factors, temperature and age, designed to account for their effects in all tested combinations. Symbols (+) or (—) indicate direction of effect of preceding factor on the character.

schemes that have been presented in previous papers (WRIGHT 1927, 1941a, b, 1942, 196a) in the attempt to represent as simply as possible the observed interaction effects. Any such scheme is necessarily tentative since there are points at which there is no real basis for choice between alternatives. It should be noted that the purpose has been to present possible sequences of interactions, irrespective of their sites of action in contrast with a scheme of color factors of the mouse given by MARKERT and SILVERS (1956). An arrow in the diagram may trace to a reaction in a pigment granule from a gene product from the nucleus of the same cell or one from a neighboring cell, or one from a cell in a remote part of the body; it may trace to a change in the external environment.

For simplicity no distinction is made between the genes represented by the usual symbols and their primary products. Unanalyzed arrays of genes are represented by Σ. Thus, $\Sigma(So)$ refers to the genetic factors for sootiness of yellow. It is convenient in many cases to use M (modifier) followed by the symbol for the gene, the main effect of which seems to be modified. Thus, $\Sigma(Ms)$ represents the array of genetic modifiers of spotting (ss). This is done even though the gene may be treated as an amorph (e.g., $\Sigma(Mp)$). The symbols $\Sigma(Leu)$ and $\Sigma(Lph)$ are used for factors that limit the amounts of eumelanins and phaeomelanins, respectively, and $\Sigma(Db)$ is used for residual factors that affect the dinginess of

brown. Numbers are associated with many of the processes for convenience of reference. The arrows indicate the directions and relative productivities of processes. Joint processes, in contrast with additive ones, are indicated by connecting the arrows by an arc. An arc at the divergence of arrows indicates alternative or competing processes. A reversed arc beyond an arrowhead indicates that the process does not contribute to color. It will be well to discuss the scheme briefly here although the justification for some of the points comes later.

Various processes that may lead to death or reduced vitality of pigment cells are represented at the left side of Figure 3, (1) to (4). The action of the *e* locus determines between a predisposition (6) toward eumelanic differentiation (*E*) or (5) toward phaeomelanic differentiation (*e*) or a mosaic (e^p), the latter subject to the effects of spotting and other modifiers. The phaeomelanic predisposition may be reversed (10) in cells under certain genetic and environmental conditions that lead to sootiness in predominantly phaeomelanic fur. The eumelanic predisposition (including tendency to sootiness) may be reversed (8) in a phase of the hair cycle by gene *A* or its feebler allele A^r (a gene introduced into the guinea pig from the Brazilian cavy, *Cavia rufescens*) (DETLEFSEN 1914; WRIGHT 1916).

It is postulated that the amounts of the primary products of the *c* alleles are in the order $C > c^k > c^d > c^r > c^a$ for reasons that will be brought out later. It is further postulated that in cells with phaeomelanic differentiation (7) something combines (13) with products of *C* (in excess and therefore dominant), c^k or c^d but that the products of c^r and c^a are specifically incapable of this reaction. Next it is postulated that c^d product while less in amount that c^k product has a specificity that enables it to take part in this reaction at a higher rate and thus to produce more yellow pigment (in adults). The term mixomorph was suggested in earlier papers (WRIGHT 1941a,b) for an allele that produces relatively abundantly a product of relatively low efficiency. The allele c^k may thus be described as a mixomorph relative to c^d with respect to yellow. STERN (1943), who has applied a similar two dimensional theory to the actions of multiple alleles in Drosophila, justly points out the advantages of a two dimensional nomenclature. We might describe c^k as a hypoefficient hypermorph relative to c^d in this case, or conversely, c^d as hyperefficient hypomorph relative to c^k. Alleles c^r and c^a behave as if amorphs with respect to yellow, but, as they produce products that are revealed in other cases, it seems better to describe them as hypomorphs of zero efficiency in this case.

The phaeomelanic enzyme precursor is represented as acting jointly with products of *F* (at (14)), or *f* (at (15)), and the product, above a certain threshold (17), as acting jointly (18) with something that is limited ($\Sigma(Lph)$) (16) to give the phaeomelanic enzyme system.

In cells with eumelanic differentiation, something combines with the *c* products at (19) to give a eumelanic enzyme precursor (*c-eu*). It is postulated, however, that this competes (20) with a feeble yellow precursor (*c-ph*). As *C* is always dominant, its product is assumed to be always in excess. It is assumed that the c^k product is apportioned largely to the eumelanic reaction while c^d product,

because of its specificity, is apportioned largely to the phaeomelanic reaction. All of the products of c^r and c^a that escape destruction are available for the eumelanic precursor. The latter must combine (at (21)) with P (or p^r an allele that differs only in its reduced eye pigment (ILJIN 1926a)) in order to be of high enough efficiency to produce dark sepias or dark brown. There is less production of eumelanin in the hair with c^dP than with c^rP, whether because of reduced quantity at this level or lower efficiency or both. Thus, c^r behaves as a hyperefficient hypomorph relative to c^d in this case. In the eyes of the same animals, on the other hand, c^d produces more eumelanic pigment than c^r, and the latter behaves merely as a hypomorph. There seems to be no competition with a yellow process in the eyes even in the most favorable cases as in pink-eyed yellows (cf. GREGORY 1928 and MARKERT and SILVERS 1956 with respect to the guinea pig and mouse, respectively). The F product behaves as a feeble substitute for P (reaction (22)) permitting the development of pale sepia or pale brown in the absence of P. In this case, the pale yellowish sepia of EBc^dc^dppF with much underlying phaeomelanin contrasts markedly with the pale slaty sepias of $EBCppF$ and EBc^kc^kppF and the neutral gray of EBc^rc^rppF (WRIGHT 1960b). The latter, however, has very little sepia pigment, less than with c^dc^d, and, thus, in it c^r behaves merely as a hypomorph. Under the scheme of Figure 3, it must be postulated that c^r has a specific property that applies to c^r-*eu* as well as to its primary product that makes it barely capable of combining with F product, or else of producing an inefficient product with it (in contrast with its behavior with P product). In an earlier scheme (1927) this double negative interpretation of the inferiority of c^r to c^d in pale sepias was avoided at the expense of complexity elsewhere. The amount of pigment in pale sepias or pale browns is subject to profound modification, represented here as due to action of modifiers $\Sigma(Mp)$ on reaction (22). In the previous paper (WRIGHT 1960) these modifiers were represented as additional feeble substitutes for P. This has the disadvantage that one would expect considerable pale eumelanin in the absence of both P and F unless the threshold were higher than it actually seems to be. The present scheme also differs from the preceding in representing f as responsible for the feeble yellow of *eeff* instead of attributing this to residual heredity and treating f as an amorph. We have also here represented f as a very feeble substitute for P, and F (23) responsible for the traces of eumelanin that are formed in pale brownish creams *ECppff*. The present scheme gives a relatively simple interpretation of the paradox that there is little or no underlying yellow in pale sepias *ECppF*, an approach to pure pale yellow in *ECppff*, considerably more yellow admixed with very pale sepias in Ec^dc^dppF but neither yellow nor sepia, leaving pure white, in Ec^dc^dppff. We may suppose that the *C-eumelanic* process prevails almost completely over the *C*-phaeomelanic process in competition for F while in the case of the c^d-eumelanic and c^d-phaeomelanic process (both weaker than the preceding), the latter tends to prevail. On the other hand, in the absence of both P and F, the *C*-eumelanic process gives a product of such low efficiency with f that even though competition leaves little of the joint *Cf*-phaeomelanic product this is efficient enough to give more yellow than the eumelanin from the *Cf*-eumelanic product. Finally both c^df-

eumelanic product and $c^d f$-phaeomelanic product may be supposed to fall below the thresholds at (24) and (17) and so give no pigment of any sort.

The *CP*, *CF* and *Cf* eumelanic products are represented in Figure 3 as acting jointly with one or the other of two limiting substances differentiated (at (25)) by genes *B* and *b*, respectively. The upper limit for the *B* substance is represented as affected by modifiers $\Sigma(Leu)$ at (26) and that for the *b* substance, some 50 percent lower than the preceding, is affected by these modifiers at (27). The effects of the products of *P* with the higher *c* alleles are supposed to be somewhat damped by the ceiling imposed by *B* and $\Sigma(Leu)$ at (26) and very much damped by the much lower ceiling imposed by *bb* and $\Sigma(Leu)$ (at 27). On the other hand, the *F* and *f* products, even with *C*, are so far below the ceiling in either case that there is substantially no damping or difference in amount of pigment.

It is postulated further that excess product of *CP* and *CF* over that required to saturate the *bb* limit (and in extreme cases the *B* limit) tends (31) to destroy the product at (29) or (30) subject (28) to various sorts of modifiers $\Sigma(Db)$, etc. IBSEN's gene *W*, absent from my colony, is required for any reduction (dinginess) of black.

Temperature and age effects: Considerable light on the physiology of the changes is thrown by experiments conducted by WOLFF (1955) in the same colony. He made reflectionmeter readings of animals at about one and one half months of age that still carried their initial pellages. Some were plucked on the rump and exposed to either 16°C or 32°C for 30 days when a reading of the new pellage was made, and others were left unplucked for 30 days at these same temperatures before making a second reading. It was found that there was no significant difference in the subcutaneous temperature whether hair was present or not (33.3°C at 16°C, 38.2°C at 32°C, rectal temperature 39.1°C). New hair grew at the rate of 0.5 mm per day. Hair of the second pellage was beginning to show slightly above the skin at the time of plucking. Such hair was not removed by plucking with the consequence that some of the new hair had been exposed to relatively low temperatures at its tip even if exposed to 32°C for 30 days before the second reading.

There were significant differences in many cases. It is desirable for the present purpose to transform the average reflectionmeter reading, as reported, into the indices used here. This is done in Table 18 for the plucking experiments which include most of the significant results. The average indices before and after exposure, and all of the differences are given for each temperature. WOLFF's paper must be consulted for the level of significance of the differences with each of the three filters that were used. The values of t from the amber and green filters were usually in good agreement while that from the blue filter was usually, but not always, somewhat less. We have indicated somewhat roughly the significance of the differences between the values before and after exposure by a single asterisk in parentheses if WOLFF found significance at at least the .05 level in only one filter, by two asterisks if there was significance at the .01 level in at least two of the three tests and one asterisk without parentheses in intermediate cases.

The dark sepia series (*EBP*) show rather consistent results as brought out in

TABLE 18

Results of transforming reflectionmeter readings described by WOLFF *into indices in cases in which comparisons can be made between areas plucked (at 1½ months of age) and subjected for 30 days to 16°C or to 32°C*

Yellow (*ee*)	T	No.	Index Before	Index After	Δ	Dark sepia (*EBP*)	T	No.	Index Before	Index After	Δ	Misc.	T	No.	Index Before	Index After	Δ
CF	16°C	25	16.8	14.9	−1.9*	*C*	16°C	9	36.1	40.5	+4.4*	*EBCppF*	16°C	12	15.2	10.9	−4.3**
	32°C	25	17.4	15.8	−1.6**		32°C	9	37.5	38.9	+1.4		32°C	12	13.5	9.3	−4.2**
	Δ		−0.6	−0.9	−0.3		Δ		−1.4	+1.6	+3.0		Δ		+1.7	+1.6	−0.1
c^kc^kF	16°C	6	11.0	11.6	+0.6*	c^kc^r	16°C	3	36.4	42.3	+5.9*	EBc^rc^rppF	16°C	7	6.1	8.3	+2.2*
	32°C	6	10.1	10.9	+0.8		32°C	4	36.0	38.9	+2.9		32°C	8	6.8	7.0	+0.2
	Δ		+0.9	+0.7	−0.2		Δ		+0.4	+3.4	+3.0		Δ		−0.7	+1.3	+2.0*
c^kc^dF	16°C	4	12.3	13.7	+1.4	c^dc^r	16°C	4	34.2	41.0	+6.8**	*EbbCPp*	16°C	12	28.0	29.8	+1.8
	32°C	3	11.5	12.6	+1.1		32°C	3	35.3	40.0	+3.7*		32°C	12	28.1	31.8	+3.7*
	Δ		+0.8	+1.1	+0.3		Δ		−2.1	+1.0	+3.1		Δ		−0.1	−2.0	−1.9
c^kc^aF	16°C	8	7.0	7.8	+0.8*	c^dc^a	16°C	8	25.7	36.8	+11.1**	*EbbCppF*	16°C	16	13.8	13.7	−0.1
	32°C	9	6.7	6.6	−0.1		32°C	5	25.5	29.1	+3.6*		32°C	16	13.9	12.2	−1.7**
	Δ		+0.3	+1.2	+0.9		Δ		+0.2	+7.7	+7.5**		Δ		−0.1	+1.5	+1.6*
c^dc^dF	16°C	14	9.7	11.9	+2.2**	c^rc^r	16°C	11	35.1	40.3	+4.2**	*ECppff*	16°C	12	3.2	2.6	−0.6*
	32°C	15	9.6	9.7	+0.1*		32°C	11	36.1	38.0	+1.9*		32°C	11	3.8	2.1	−1.7**
	Δ		+0.1	+2.2	+2.1*		Δ		0.0	+2.3	+2.3**		Δ		−0.6	+0.5	+1.1*
c^dc^raF	16°C	16	5.3	8.5	+3.2**	c^rc^a	16°C	13	29.9	36.4	+6.5**	eec^rc^aP	16°C	3	0.0	2.1	+2.1**
	32°C	16	5.5	6.0	+0.5		32°C	12	29.5	34.4	+4.9**	(sooty)	32°C	4	0.0	0.1	+0.1
	Δ		−0.2	+2.5	+2.7**		Δ		+0.4	+2.0	+1.6		Δ		0.0	+2.0	+2.0**
Cff	16°C	6	9.5	7.3	−2.2*	c^ac^a	16°C	9	0.0	+3.9	+3.9**						
	32°C	4	11.5	6.8	−4.7*		32°C	16	0.0	+0.3	+0.3**						
	Δ		−2.0	+0.5	+2.5*		Δ		0.0	+3.6	+3.6**						

See footnotes Table 7.

† Combination of classes kept separate by WOLFF are made in several cases. These indices cannot be compared fully with those described elsewhere because they were made on the rump instead of the middle of the back as well as because of difference in age. Differences are, however, comparable to those in Tables 7 to 11. In the case of c^ac^a, seven albinos which did not darken at 16°C and which were quite certainly not *EBP* are excluded. It is not certain how many of 10 at 32°C were *EBP* since none darkened more than a trace.

WOLFF's analysis. The new pellage was in all cases darker than the old at both 16°C and 32°C, but the darkening was in all cases greater at the lower temperature. In the albinos there was appreciable darkening only at 16°C in agreement with the long-known effect of low temperature in this case and in the similar case of the Himalayan rabbit (SCHULTZ 1918; ILJIN 1926b; WRIGHT 1927). Apart from this, the intensity at 16°C was significantly greater than that at 32°C only in the cases of $c^d c^a$ and $c^r c^r$. There was significant darkening at 16°C in all cases, though somewhat doubtfully in the intense blacks (*EBCP*). The relatively slight darkening at 32°C (greatest in $c^r c^a$, $c^d c^a$, $c^d c^d$ and $c^d c^r$) may well have been merely the effect of low temperature on the tips of the hairs that had already begun to develop at the time of plucking.

As to the physiological nature of the process, DANNEEL and SCHAUMANN (1938) have attributed the similar process in the Himalayan rabbit to thermolability of the enzyme produced by the albino allele that characterizes them. Our hypothesis in the case of the guinea pig is that the product of c^a is completely destroyed (process (12) in Figure 3) at body temperature but that some escapes at the lower temperature of the skin after birth, especially in peripheral regions (ears, feet, nose), that the c^r product is greatly reduced at body temperature (40 percent) and that the c^d product is reduced somewhat less (some 30 percent). The C and c^k products seem to have been reduced much less since the slight darkening effect of low temperature indicated by WOLFF's results is overbalanced at six months by a temperature-independent weakening of pigmentation. On the other hand, there is clearly some damping of the effects of the c compounds by an upper limit suggesting that the smallness of the low temperature effects on C and c^k may be due at least in part to this. The smaller amounts of darkening of the c^d compounds with only three or four plus modifiers in contrast with one or two (shown in Table 2) point in this direction. With darkening restricted by a ceiling, the observed reduction in the C and c^k compounds at six months must be due to lowering of the ceiling (process (26), Figure 3) rather than to a tendency to proportional reduction of all c compounds. The best evidence that there is actually much less low temperature effect on C and c^k than on the lower alleles comes from consideration of the yellows.

WOLFF found a highly significant reduction in the intensity of red (*eeCF*) at both 16°C and 32°C. We may conclude that the 30 percent reduction, shown in Table 17 and Figure 2, is due to a weakening of the process of forming yellow pigment that occurs within one half a year after birth, independently of temperature (processes (14) and (15) in Figure 3) and that this occurs to a significant extent by two and one half months. The reduction in the c^k yellows ($c^k c^k$, $c^k c^r$, $c^k c^a$), shown in Table 17 and Figure 2, is due presumably to a similar process that occurs so slowly that it was not revealed in WOLFF's experiment. The increases in intensity of the c^d yellows ($c^d c^d$, $c^d c^r$, $c^d c^a$) of Table 17 and Figure 2 correspond to highly significant deepening of color found by WOLFF in these same genotypes at 16°C but not 32°C. This reaction, thus, must be considered as due to low temperature (process (12) in Figure 3). WOLFF found a slight, but possibly significant, reaction of this sort with $c^k c^a$. We may interpret these results

as implying that in the case of c^k the weakening of pigmentation with aging ultimately dominates over a slight stimulatory effect of lower temperature that occurs at birth while in the case of the c^d yellows, the latter process completely dominates over any aging effect. It should be noted that in referring to an aging effect it is not meant to imply an effect of old age. Guinea pigs do not reach their full weight until they are more than one and one half years old and, thus, are very immature physically at one half year. It is clear from the lightening of c^kc^{ra}, with only 17 percent as much pigment as *C* yellows, that in this case the process is not a mere lowering of an upper limit but a more or less proportional dilution at all levels.

The greater intensification of c^dc^{ra} and to some extent of c^dc^d with three or four plus modifiers, instead of one or two, is not easy to account for. It does not occur in c^kc^{ra} yellows and is opposite in direction to the effects in c^d sepias. A specific interaction effect of *dm* and *si* with c^d (that permits excess fading to offset the low temperature effect) seems required.

The difference between c^k and c^d yellows with respect to direction of change after birth requires consideration of some results reported by IBSEN (1932). In a list of minor variations in his colony, he included a fading after birth of certain light yellows (eec^dc^a) which he attributed to a gene that he named *f*. No data were given. This gene was clearly not the same as that which I had designated *f* (WRIGHT 1923) since eec^dc^aff in my terminology was found to be white or near-white at birth (WRIGHT 1927) and to have other major effects, not referred to by IBSEN. To avoid confusion, we will use f_I here for IBSEN's gene.

This gene was clearly not present in my stock unless it can be supposed that it was very strongly associated with c^k and its dominant allele, F_I, with c^d. It would, however, require complete or very nearly complete linkage for such an association to have been fully maintaind from 1916 to 1954 in the face of the deliberate attempt to make the genetic backgrounds of c^k and c^d alike by mating c^kc^a with albino segregants from $c^dc^a \times c^ac^a$ and *vice versa* (cf. WRIGHT 1925).

A good example of this association is provided by two late matings, YC 70 and YC 71. The former was of the type $eec^ac^aFF \times eec^kc^dFF$. The young were graded visually at birth without knowledge of whether they were c^kc^a or c^dc^a. Fourteen of them were mated with albinos transmitting *E* or e^p to test the genotype (Ec^kc^aP being unmistakably darker sepia than Ec^dc^aP). Eleven turned out to be c^kc^a and only three c^dc^a. All of the former became paler (mean at birth, grade 4.55, mean as adults 3.70). One of the c^dc^a's remained unchanged, but the others deepened in color (mean of three at birth, 4.00; as adults, 4.67). Mating YC 71 was of the type $eec^kc^aFF \times eec^dc^aFF$. The sire ($c^kc^a$) was of grade 5 at birth, changing to 4 later, while the dam (c^dc^a) also of grade 5 at birth, became of grade 7. The three young that proved to be c^kc^a on test all became paler (4.67 at birth, 4.00 later). All of those young that proved to be c^dc^a deepened (also 4.67 at birth but 6.00 later). Altogether, the 14 tested c^kc^a from both matings decreased an average of 0.81 ± 0.10 while the six tested c^dc^a deepened by 1.00 ± 0.30.

The marked difference in behavior of the c^k and c^d yellows exhibited in Tables 1 and 7 confirms the earlier results, but here applies to the last three years of

the colony after abundant opportunity for randomizing any linked modifiers over a period of nearly 40 years.

Thus, the available evidence all indicates that the tendency to become paler cannot be dissociated from c^k nor the deepening tendency from c^d. It is simplest to consider these to be properties of the two alleles themselves rather than of linked modifiers. IBSEN used only one symbol, c^d, for a c allele found in dilute yellows in his stock. It seems possible that his observation of segregation of fading and nonfading within his yellow strain was actually based on segregation on c^k and c^d. He refers to matings of $c^dc^af_lf_l$ with dilute blacks (EBc^dc^dP) but does not give any details that would indicate whether both c^k and c^d were present in the descendants. He notes, however, that the fading could not be transferred to blacks to produce a "blue." This is not surprising if the gene transferred was c^k which dilutes black much less than does c^d.

On bringing together the results from the dark sepia and F yellows with respect to the effect of temperature on c compounds, it is evident that the order of sensitivity, from greatest to least, is $c^a > c^r > c^d > c^k$ among the lower alleles. This indicates that the amounts of product are in the opposite order. The complete dominance of C (which applies within all of the color series) and incomplete dominance in all other cases where there is a difference, indicate that only C produces an excess of product with respect to the substrate. Thus, the primary quantitative order may be taken as C,c^k,c^d,c^r,c^a from highest to lowest, and only the deviations from this that occur in particular cases require explanation in terms of differences in efficiency in some sense.

In the case of *eeCff*, WOLFF found considerably more reduction of intensity at both 16°C and 32°C than with *eeCF*. While there was greater reduction at the higher temperature, this was of doubtful significance. It is probable that the 60 percent reduction in intensity shown in Table 17 and Figure 2 at one half year is due to a temperature-independent weakening of pigment production that is greatly enhanced by replacement of F by f. The pertinent process is located at (15) in Figure 3.

With c^kc^kff, c^kc^dff *and* c^dc^dff, the small amount of yellow pigment at birth (about five percent of that in reds) is wholly or almost wholly lost in adults. This and the complete or nearly complete whiteness of the heterozygotes of c^k or c^d with c^r or c^a indicate a threshold in the yellow process of about five percent of intense red (at (17) in Figure 3).

The pale sepias, *EBCppF*, showed approximately equal and highly significant decreases in intensity at 16°C and 32°C in WOLFF's experiment. We accordingly interpret the drastic and long continuing decreases in intensity that occur normally (Tables 5 and 10) as the result in the main of a temperature-independent weakening of process (22). Process (23) probably also falls off but is shown by the feebleness of eumelanic pigmentation in *ECppff* to be of little importance. The decreases in all other c compounds except c^rc^r and c^rc^a can be interpreted in the same way. In the case of c^rc^r, however, the increase in intensity that WOLFF found at 16°C indicates that even the drastic weakening of pigmentation with age in pale sepias can be overbalanced by the strong response to low temperature characteristic of c^r.

WOLFF's results with intense brown, *EbbCPp*, are the most paradoxical that he obtained, from the standpoint of the normal reduction in intensity shown in Tables 4 and 9. He found intensification at both 16°C and 32°C, and this was significant at the higher temperature in contrast with all other cases.

It should be said, however, that strong intensification of brown has often been observed in new hair of young animals that have lost much of their initial coat. There is a process here that requires further study.

The decreases in intensity in the preceding cases, except for the slight one in blacks, have appeared to apply more or less proportionately to all *c* compounds, after allowing for the release at birth from destruction by high temperature. The decreases in the dark browns are clearly not of this sort. As often noted, comparisons of the relative intensities of the *c* compounds of dark browns with those of dark sepias indicates that the former are damped much more on approach to a ceiling, by some limiting factor. The adult results suggest a lowering of this ceiling from 50 to about 38 or 39 by a process located at (27) in Figure 3. None of the *c* compounds in adults can be distinguished with any confidence except $c^a c^a$ (which shows the same sort of peripheral darkening as in albinos of the dark sepia series). All of these except $c^d c^a$ and $c^r c^a$ are brought down to the new ceiling, while $c^d c^a$ and $c^r c^a$ rise toward it (Figure 1) presumably because of the low temperature effect.

Brown reaches its maximum intensity at birth with *CPpff*. As has been shown, the production of *C*, *P* and *F* in excess of that required to reach the ceiling leads to inhibition (dinginess) which is greatest with *CCPPFF* (WRIGHT 1947, 1960a). With lowering of the ceiling before six months of age there is enough excess of *CP* and *CF* products to reduce dingy grown males some 23 percent below the new ceiling (Table 4). The females, however, tend to lose their dinginess and become on the average 12 percent more intense than at birth, though still usually below the ceiling at their age.

This difference between males and females is interesting as it is the only known sex difference in color in guinea pigs except for a slightly greater amount of white in spotted females (*ss*) than in spotted males of the same inbred strain (WRIGHT 1926). WOLFF (1954) made experiments with castrated males and ovariectomized females, both with and without injected estrogen. The classes without androgen, whether with or without estrogen, darkened while the normal males (the only class with androgen) tended to lighten slightly. The difference between the males and the others as a group was significant at the .01 level. The available number of dingies (strain SH) was unfortunately inadequate for further testing of androgen, but it was concluded that it was a reasonable working hypothesis that testicular androgens prevent a loss of dinginess that otherwise tends to occur. This loss of dinginess can hardly be due to a higher ceiling since this is not apparent in nondingy females. Androgen is represented in Figure 3, associated with spotting, IBSEN's gene Whitish (*W*), and other modifiers in affecting the dingy process.

Pale browns (*EbbCppF*) showed no appreciable change at 16°C in WOLFF's experiments but a significant lightening at 32°C. Even the latter is much less than the lightening observed at both temperatures in pale sepias.

By six months of age, however, all *c* compounds except c^rc^r and c^rc^a show decreases much like those shown by pale sepias while c^rc^r and c^rc^a at least hold their own, presumably because of the strong low temperature effect on c^r. The same effects at (22) and (23) are to be expected as in pale sepias. The apparently smaller amount of decrease needs further study.

The pale brownish creams (*ECppff*) gave results rather similar to those given by the pale browns in WOLFF's experiments: a small but significant decrease at 32°C and no significant change at 16°C. Again, however, there is great reduction at one half year of age, though not as great a one as in the similarly pale yellows of genotype eec^kc^kff, which as noted becomes pure white or very nearly so. The processes in Figure 3 that should be involved are (23) for the feeble eumelanic component and (15) for the phaeomelanic one. In the earlier records, *ECppff* appeared to be indistinguishable from eec^kc^kff at birth, and it may appear that there should be equal reduction by weakening of process (15). In the case of eec^kc^kff, however, there is no eumelanic process at all, and weakening of (15) has its full effect. In the case of *ECppff* the phaeomelanic process is assumed to be very much reduced by the presence of eumelanic differentiation. The *C* eumelanic and *C* phaeomelanic processes are represented in the diagram as competing for *f*. If process (23) is weakened after birth, process (15), relieved to some extent from competition, would not decrease as much as where process (23) is not occurring at all. This could account for the fact that *ECppff* fades less than eec^kc^kff.

WOLFF's results bring out one type of change that has not been dealt with so far. In eight of 50 yellows of genotype *eeBPF* (two *C*, one c^kc^d, two c^kc^a, one c^dc^d, one c^dc^r, one c^dc^a) excluded from his tables, and in all three of genotype $eeBc^rc^aBF$ (Table 18) more or less eumelanin developed after exposure to 16°C. None developed after exposure to 32°C. He concluded that low temperature is a necessary but not a sufficient condition for sootiness in *ee* genotypes. It may be noted that SCHULTZ (1922) showed that the development of sootiness in yellow rabbits of genotype *eeaaBC* depends on low temperature. A somewhat similar proportion of the yellow guinea pigs carrying *P* develop conspicuous sootiness naturally after birth, and some of these were graded but all such records have been excluded from the averages for yellow in our tables. The degrees of sootiness in adults varied from a mere trace to almost the appearance of a light sepia.

According to IBSEN (1932) there is a genetic basis for sootiness. It is probable that the conjunction of suitable heredity $\Sigma(So)$ and low temperature is necessary. Its prevention by high temperature seems to require a different thermolabile enzyme than that postulated as due to the *c* alleles. This process is represented at (10) in Figure 3.

There are certain other age effects which have not been considered so far. Guinea pigs with agouti allele A^r, derived from many generations of backcrossing the Brazilian cavy (*Cavia rufescens*) to black guinea pigs, were self black at birth except for very narrow banding with yellow on the belly and nape (WRIGHT 1916). The amount of yellow increased considerably later in life. It is possible that measurement would show similar changes in the effect of the allele *A*, but this has not been tested.

The gene *gr* (for grizzling) shows a conspicuous increase in effect with age. There is no effect of *grgr* at birth in any genotype, but with aging the hairs on the back (whatever the color) gradually become white (LAMBERT 1935; WRIGHT 1947). There is often some effect of this sort in heterozygotes. Presumably there is premature death of the pigment cells. This progressive grizzling (process (4), Figure 3) contrasts with the effects of *Sisi* and *sisi* (silvering) which are manifest at birth and not progressive.

Finally, we may note an effect of increasing age of the mother. Analysis of spotting in an inbred strain revealed that as females became older the amount of white in the spotting pattern of their offspring became significantly greater. In this case there is no change in individuals (WRIGHT 1926).

SUMMARY

There are changes after birth in the intensities of the various coat colors of the guinea pig that differ in direction and magnitude, and, in some cases, there are changes in quality. At least ten different processes must be involved.

The outstanding changes in the dark sepia series (*EBP*) are the darkening of ears, feet, nose and sometimes backs of albinos (c^ac^a), the strong general darkening of the lighter sepias (c^rc^a and c^dc^a) and the moderate darkening of the sepias that involve only c^r and c^d. These effects are attributed to thermolability of the products of the *c* locus (100, 40 and 30 percent destruction of the products of c^a, c^r and c^d, respectively, before birth as compared with after birth). There is much less destruction of the products of *C* and c^k, and the genotypes carrying these become slightly lighter at one half year of age because of a slight lowering of the upper limit.

In the pale sepias (*EBppF*) there is a marked and long continuing reduction in intensity (50 to 60 percent by one half year) that is independent of temperature and is attributed to the eumelanic processes that occur in the absence of *P*. These are overbalanced by the low temperature effect only in the very pale sepias of genotypes c^rc^r and c^rc^a.

In the dark browns (*EbbP*) there is a lowering of the upper limit, already 50 percent lower with *bb* than with *B*, by an additional 12 percent independently of temperature. The albinos darken similarly to those with *BP*. The light browns (c^rc^a, c^dc^a) darken to almost the same intensity as that to which the higher *c* compounds are reduced by the lowering of the ceiling. The limiting factor seems to be fully saturated in genotype *EbbCPpff*. Excess *CP* and *CF* product leads to inhibition (dinginess) and intensities far below the ceiling. There is further reduction after birth in dingy males, but the dingy females tend to become more intense though those with *CCPPFF* are still lighter than those with *CPpff*.

The pale browns (*EbbppF*) behave similarly to the pale sepias for presumably the same reason. The amount of fading of the *c* compounds after birth (other than c^rc^r and c^rc^a which again intensify slightly) seems to be somewhat less than in the pale sepias.

The yellows (*eeF*) that carry *C*, c^kc^k, c^kc^r or c^kc^a fall off some 20 to 30 percent in the first half year, independently of temperature. This cannot be due to lowering of the ceiling since c^kc^r and c^kc^a have only 18 percent as much pigment as *C*.

This is attributed to the phaeomelanic process that involves *F*. The genotypes with c^d, on the other hand, tend to increase in intensity because the low temperature effect on c^d overbalances any tendency to fade. No yellow at all is produced at any age with c^rc^r, c^rc^a or c^ac^a.

The fading yellows (*eeff*) show a much more drastic reduction in intensity after birth (some 50–60 percent) that is again independent of temperature and is attributed to the phaeomelanic process that involves *f*. The extremely pale yellows c^kc^k, c^kc^d and c^dc^d are all reduced to white, presumably by the same process with no apparent offsetting by the low temperature effect on c^d. The heterozygotes of c^k and c^d with c^r or c^a are white or near-white at birth and lose whatever traces of yellow they may have.

All of the yellows (*eeF* or *eeff*) are subject to a whole different process under certain conditions which include low temperature. Many animals that are pure yellow at birth develop sootiness of the type expected from the genes that affect the eumelanic process.

The pale brownish creams (*ECppff*) are greatly reduced in intensity by one half year but not as much as pale yellows eec^kc^kff of similar intensity. The effects are independent of temperature, and that on yellow is presumably the same as in *eeff* yellows but is buffered by reduction in the competing *Cf* eumelanic process.

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A COMPARISON OF THE EFFECTS, IN COMPOUNDS, OF SEVEN GENETICALLY SIMILAR LETHAL T ALLELES FROM POPULATIONS OF WILD HOUSE MICE[1]

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POPULATIONS of wild house mice in different parts of the United States are frequently polymorphic for variant alleles (*t* alleles) at the complex locus, *T*. One step in the analysis of any such polymorphism is to determine whether the variant alleles found in different populations are nonidentical and, thus, probably due to separate events, or whether they can be shown to be similar or identical and, thus, possibly traceable to a common origin.

In previous papers (BENNETT and DUNN 1958; BENNETT, DUNN and BADENHAUSEN 1959) we have described the methods by which lethal *t* alleles from different wild populations have been classified into two groups. Lethal alleles are assigned to different groups when some of the compounds between them are viable until birth. We have taken such evidence of complementary interaction as a criterion of nonidentity for the alleles concerned. The alleles in heterozygotes which when crossed fail to produce such compounds viable at birth are tentatively assigned to the same group. Since absence of compounds at birth is not a decisive criterion of identity of the alleles concerned, the homozygous effects of alleles assigned to the same group are then compared by embryological methods. Such comparisons have been made for four (including t^{w20}) group 2 lethals (BENNETT, BADENHAUSEN and DUNN 1959), and for five group 3 lethals (BENNETT and DUNN 1958). (Group 1 consists of indistinguishable viable alleles). The conclusions were that members of each group were indistinguishable from each other when judged by effects on homozygotes. Group 2 lethals were however clearly distinguishable from those of group 3.

Members of these two groups of lethals are unequally represented in the wild populations which we have sampled. We have found group 2 lethals in three populations (t^{w1}, New York 1; t^{w3}, Mystic, Conn.; t^{w12}, Marin County, California) and group 3 lethals in nine populations (t^{w5}, New York 2; t^{w6}, Sarasota, Florida; t^{w10}, Clark Fork River, Montana (collected by Miss VIRGINIA VINCENT and reported here for the first time, but not yet studied embryologically); t^{w11}, Storrs, Conn.; t^{w13}, El Paso, Texas; t^{w14}, Norwich, Vermont; t^{w15}, Lawrence, Kansas; t^{w16}, Norwich, Vermont; t^{w17}, Tucson, Arizona. Since t^{w14} and t^{w16} came from different stations on the same farm, they may have come from the same population. We may say then that group 3 lethals are found in nearly three

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times as many locations as group 2 lethals. This may be due to chance. It does, however, suggest caution in drawing conclusions as to similarity or identity among the alleles of a group so widely disseminated in different habitats throughout the United States.

Embryological examination revealed that the five alleles studied from this group were not entirely consistent in the degree of their effects. One of them, t^{w6}, especially, seemed to have somewhat more severe and earlier effects in homozygotes than the other members of the group. We have, therefore, studied the many members of this group more thoroughly and extensively by methods which we consider to be more sensitive for disclosing differences between them. In essence, we sought for evidences of partial complementarity amongst the alleles of this group by examining the compound embryos in all of the combinations that could be produced by crosses between stocks containing eight lethal alleles belonging to this group, including the five previously studied in homozygous form.

MATERIALS AND METHODS

The eight alleles (omitting t^{w10}) were carried in our laboratory as tailless balanced lethal lines of the type T/t^{w}, each of which had been derived from the wild population indicated by: (1) the initial crossing of the normal tailed ancestor carrying the t allele to an inbred Brachy laboratory stock, (2) two or three subsequent backcrosses of tailless animals derived from the first cross to the Brachy stock, and (3) three or more generations of inbreeding of the descendants of a pair of tailless animals derived from the last backcross.

To obtain for study embryos heterozygous for two different t^{w} alleles, crosses were made in every possible combination between tailless balanced lethal stocks. Our previous study on homozygotes had indicated that the presence of T/T embryos did not interfere with the identification of t^{w}/t^{w} homozygotes since T/T homozygotes are rarely found (because of the high proportion of t^{w} gametes produced by males) and died at a much later stage showing a different syndrome of abnormalities. Where possible at least six litters of embryos were obtained from each cross; they were timed by the conventional vaginal plug method, and pregnant females were sacrificed at appropriate times to give embryos (for study) which were between seven and ten days postfertilization. From our previous studies on homozygotes, we could expect that compound t^{wx}/t^{wn} embryos, if they were similar to the homozygotes, would already have detectable abnormalities at the beginning of this period and would have undergone maximum differentiation (and often the deterioration and death which always follows) by the end of it. All the embryos obtained were examined histologically; the details of the procedure followed are given in full in our previous paper (1958).

RESULTS

The first and rather broad test for the existence of differences among the alleles studied was carried out in the following way. As stated above, the embryos were obtained during a time period in which we expected to be able to

recover and identify the great majority of embryos abnormal because of genotypic constitution t^{w2}/t^{w_n}, if the compound behaved in a similar way to t^{w2}/t^{w2} and t^{w_n}/t^{w_n}. If the t^{w2}/t^{w_n} compound behaved differently, i.e., was more viable or more normal morphologically, we expected to detect it in one of two ways: (1) gross deviations from the morphological pattern typical of homozygotes, or (2) significant departures from the segregation ratio expected; most probably this would be manifest as a deficiency of embryos scored as "abnormal." Table 1 gives the segregation data for the individual crosses. The embryos listed as "typical abnormals" were all similar in every way to the homozygous abnormal embryos described previously (1958) and thus did not fulfill the criterion for morphological diversity. It is clear, also, that in spite of the rather small numbers of embryos obtained from some of the crosses, there are no significantly large deviations from the expected segregation of about 0.48 t^{w2}/t^{w_n}:0.52 +/+ or T/T, a figure based on an estimated average segregation ratio in males heterozygous for these alleles of 0.95 t:0.05 T. It was immediately obvious then, that if a condition of partial complementarity did exist between any of these alleles, it was of too minor a nature to be detected by these relatively gross methods.

The material was therefore subjected to what we thought would be a more

TABLE 1

Percentages of abnormal (t/t) *embryos found in crosses among members of the* t^{w5} *group. (Expected percent of "typical abnormals" ≃ 48 percent)*

	Number of litters	Number of embryos	Number of typical abnormal embryos	Percent of typical abnormal embryos
$t^{w5} \times t^{w6}$	7	51	23	45
$\times t^{w11}$	6	47	20	43
$\times t^{w13}$	6	57	27	47
$\times t^{w15}$	7	61	27	44
$\times t^{w16(14)}$	6	43	19	44
$\times t^{w17}$	6	46	20	43
$t^{w6} \times t^{w11}$	6	43	22	51
$\times t^{w13}$	3	9	6	67
$\times t^{w15}$	5	31	12	40
$\times t^{w16(14)}$	5	35	17	49
$\times t^{w17}$	6	36	15	43
$t^{w11} \times t^{w13}$	3	25	9	36
$\times t^{w15}$	5	38	16	42
$\times t^{w16(14)}$	4	35	16	46
$\times t^{w17}$	4	27	13	48
$t^{w13} \times t^{w15}$	4	35	17	49
$\times t^{w16(14)}$	4	34	18	53
$\times t^{w17}$	3	19	7	37
$t^{w15} \times t^{w16(14)}$	5	36	15	43
$\times t^{w17}$	6	40	19	48
$t^{w16(14)} \times t^{w17}$	6	52	25	48
Totals	107	800	353	. .

delicate analysis, according to the following rationale. In our previous paper, the abnormal embryos were staged and divided into groups, not on the basis of their own morphology, but according to the developmental stage of their normal litter mates. This was necessary to permit us to form some idea of the temporal relationships between normal and abnormal development. For the purposes of this paper, however, now that we already have basic information concerning timing and morphology, and where we were interested in discriminating as delicately as possible among conceivably different types or degrees of abnormality, we staged the abnormal embryos only with reference to themselves. Accordingly, a series of stages of abnormal development was prepared, in such a way as to include as many discrete and identifiable stages as possible. This permitted the construction of a series of frequency distributions of the various stages of abnormal embryos found in each separate cross, comparable with one another, and in which differences of distribution of abnormal stages could be easily detected. A brief description of these stages will be sufficient here; a more complete exposition of the abnormal embryology is given in our previous paper on group 3 (BENNETT and DUNN 1958).

The stages used were the following: 0.—Embryo entirely resorbed. I.—Embryo exists as a nubbin of undifferentiated cells; it looks as if almost no differentiation had occurred, and as if none will. Capsule and ectoplacental area are small. II.—Embryo is a degenerate egg cylinder, with many pycnotic granules and often grossly crumpled; however, it is large and presumably will develop further. III.—Embryo is very small and lumplike, and shows little or no differentiation; it differs from Stage I only in that the capsule is larger, and the embryo looks as if growth had proceeded somewhat, before regression occurred. IV.—Embryo has infolding ectoderm, but it is small with little or no development of mesoderm; looks as if it will not develop much further. V.—Embryo is large and healthy looking, with infolding ectoderm and developing extra embryonic membranes. VI.—Degenerating lump of ectoderm with small extraembryonic membranes is the only vestige of embryo. The whole capsule is small and looks as if development will shortly stop. VII.—Large and healthy extraembryonic structures with a variable amount of surviving ectoderm on top. VIII.—Small and degenerate "extraembryonic organism." IX.—Large and healthy "extraembryonic organism." X.—Rudimentary embryo, with organization of tube or rosette-like structure in the embryonic ectoderm, with some mesoderm surrounding it; all associated with large extraembryonic structures. XI.—Most advanced differentiation. Possible neural folds, notochord, and somites.

Since in the first analysis no members of group 3 had been detected as clear-cut deviants (either in morphological pattern or in segregation) we decided to consider the alleles as a group and test one after another individually against the remainder of the group. In addition, we knew from observation that these lethal compounds developed slowly enough as to form a fairly homogeneous group at 7–8 days gestation, and also at 9–10 days.

With these ideas in mind, we prepared the histograms shown in Figure 1.

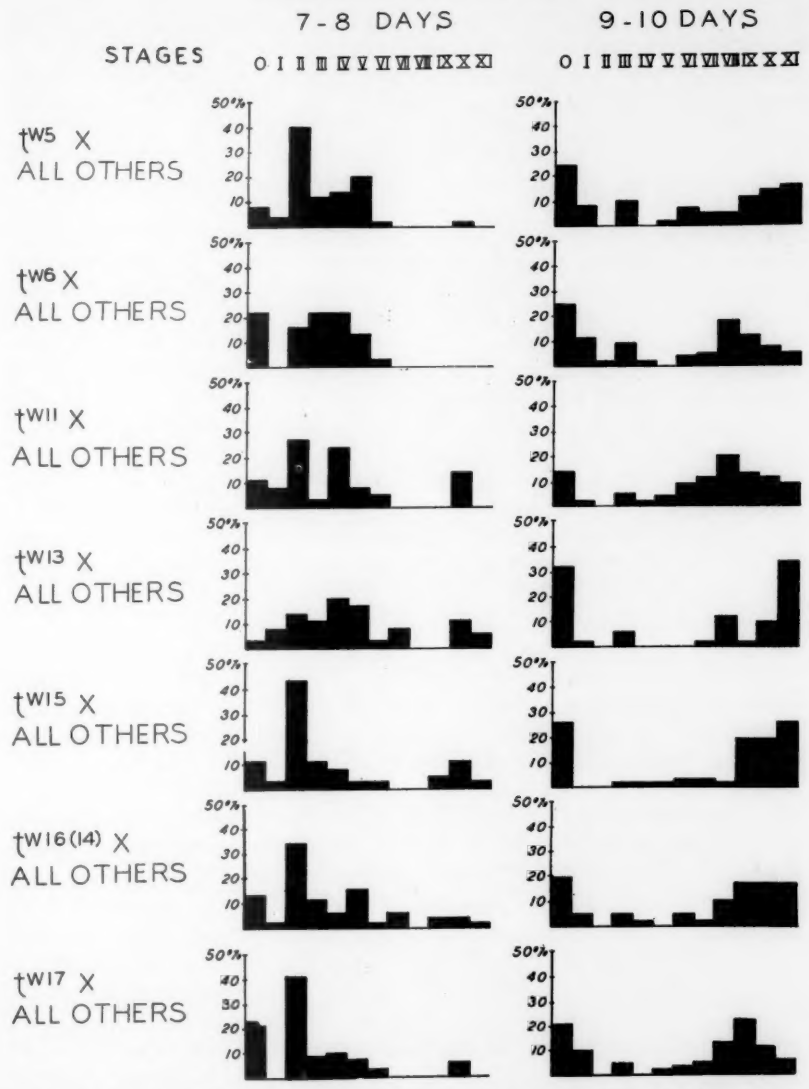

FIGURE 1.—Histograms indicate the distribution, according to stage, of lethal embryos at ages 7–8 days and 9–10 days, with each allele considered separately in compound with all other alleles studied.

If any one of these alleles had been different from the others, this difference should have been detectable in its cross with all the other alleles by a distribution of abnormal stages which was clearly different from any of the others. Inspection of Figure 1 indicates, however, that the differences in the frequency distribution of abnormal stages between crosses at 7–8 days and at 9–10 days are not large enough to give any substantial hint that the effects of any one of these alleles is different from any of the others; the only one that might be thought to be deviant is t^{w6}.

Still another method of comparison involved assigning to each stage its own number, as a "weight," since the stages had been made up as a series indicating progressively increasing life span and/or differentiation. This made it possible then to prepare an "index of abnormality" for each of the crosses. These data are presented in Table 2. Again it is evident that all the alleles behave in much the same way in compounds. For comparison with the indices of abnormality, the average litter size for all this material is presented in the last column. The allele t^{w6} deviates somewhat from the others in two ways. It has the lowest index of "abnormality stage" indicating slightly earlier attainment of the lethal syndrome on the part of any compounds in which it enters; and liters of which a t^{w6} heterozygote was one of the parents were smaller than those produced by other heterozygotes. Both of these differences may be reflections of the state of the t^{w6} balanced stock which, perhaps because of its residual heredity, has been inferior to the other stocks in its fertility and the viability of its progeny.

DISCUSSION

This detailed comparison of the effects on embryonic development of lethal alleles derived from seven different wild populations indicates that their effects in compound with each other do not differ significantly from their effects when homozygous. The differences shown at 7–8 days (Figure 1) can be interpreted as minor variations on the same theme, which among the survivors at 9–10 days show a similar and consistent pattern. We have, moreover, to consider that genetical tests had shown no evidence of complementary interaction amongst them, and that the syndrome which all exhibit is unique both in time and

TABLE 2

A comparison of the effects, in compound, of seven lethal t *alleles each derived from a different population of wild house mice*

Type of mating		Average abnormal index; 7–8 days	Average abnormal index; 9–10 days	Average litter size
t^{w5}	Mated by all other alleles	3.4	5.9	8.5
t^{w6}		2.6	5.0	6.5
t^{w11}		3.8	6.3	7.7
t^{w13}		4.6	6.5	7.8
t^{w15}		4.0	6.7	7.5
$t^{w16(14)}$		4.8	6.4	7.9
t^{w17}		3.6	5.6	7.3

manner of expression amongst the lethals so far examined in the house mouse. The alleles of this group have also been shown to share other genetical properties: all have male transmission ratios in excess of 0.9, and all appear to suppress recombination in the region to the right of the locus. We consider these resemblances to come as near to proof of identity as it is possible to reach with such material.

If these do represent occurrences of the same allele in widely separated populations, how are we to account for their disjunct distribution? Two main hypotheses suggest themselves: (1) all arose in one act of "mutation" whether this were to be considered as point mutation in the usual sense or as a rare recombination near this locus; (2) they represent recurrences of the same event. We have no direct evidence in favor of one or the other of these hypotheses. The second hypothesis, assuming repeated independent acts of mutation resulting in alleles with the same or similar properties, encounters two difficulties. One is that mutation from the wild type to a mutant condition of this locus (+/+ to +/t) must be a rare event since we have encountered no instance of it in large numbers (over 20,000) of gametes from +/+ or T/+ when tested in matings by T/+. The other is that since the only observed method of origin of new t alleles is a change in the properties of t alleles already present (T/t^n or $+/t^n \rightarrow t^x$; cf., DUNN 1956), such changes, if they were to account for the origins of the eight similar alleles described here, would all have to have occurred in populations polymorphic for a t allele and in similar ways to produce indistinguishable lethal alleles. This would require multiple repetitions of the same event. We have observed two occurrences of such a repetition in the origins of t^{w20} from $+/t^{w15}$ and t^{w21} from T/t^{w5}. Both t^{w20} and t^{w21} are indistinguishable from the previously studied alleles of group 2, and repetition of events resulting in similar alleles is, thus, not to be excluded. On the other hand, out of 22 such events which we have analyzed, 14 have resulted in viable alleles and eight in lethals, only two of which are known to be duplicates. We should, therefore, have to assume some other agency favoring the retention of similar lethals in wild populations. Since all eight lethal alleles reported here are characterized by high transmission ratios from male heterozygotes (LEWONTIN and DUNN 1960), this may be the agency leading to the retention of duplicates. Since the second hypothesis is a possible one, we may compare it with the first hypothesis.

If we assume a single origin for the eight alleles, their similarities in effects on development, on male transmission ratio and on suppression of recombination would all be explained by their common origin. We should then have to explain their distribution in widely different populations. This could be due to migrants carrying the same allele to other populations. Migration rates in this species are not well known although, in general, the range of movement of individual mice is rather restricted (BLAIR 1953). But as a commensal its mobility is likely to be increased by travelling with man. It is therefore possible that the disjunct distribution of the same allele could be due to human agencies.

In the absence of decisive proof of either hypothesis, we may suspend judgment but with the remark that fewer difficulties are encountered by the hypothesis of common origin.

SUMMARY

Eight lethal alleles at locus *T* in the house mouse, each derived from a single heterozygote $+/t$ found in one of eight different North American populations, were compared by breeding tests and by embryological study. All possible compounds proved to be lethal at similar stages and to have similar patterns of abnormality between the seventh and tenth days of development when death occurred. No constant differences between compounds and homozygotes were found.

The essential disturbances in development, shared by the eight lethals, were those identified in a previous study of five of them as follows: pycnosis and faulty growth of the embryonic ectoderm as soon as it formed; the subsequent degeneration of all actual embryonic structures, and the preservation for a short time thereafter of nearly normal extraembryonic structures.

Since the alleles are indistinguishable by genetical and embryological methods, two hypotheses to explain their disjunct distribution in widely separated populations are examined *viz.*, (1) common origin in a single mutation followed by dispersal, possibly by human agencies; (2) repeated independent occurrences of the same mutation in different populations. Although no final decision is possible from present evidence, the first hypothesis encounters fewer difficulties than the second.

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A SEX RATIO FACTOR IN THE HOUSE MOUSE THAT IS TRANSMITTED BY THE MALE[1]

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TO undertake a study of the sex ratio of mammals requires courage in the face of a vast literature that bears testimony to the capricious nature of the character. For ten years we have been studying a sex ratio factor that distinguishes two lines of mice; one line produces a consistent excess of males, the other a consistent excess of females. There have been a number of changes in environment over the years, some drastic. There have been four changes of diet, and the entire colony was moved in 1950 from the University of Saskatchewan to the University of Kansas. Subsequently there have been two changes of animal quarters. In spite of these disturbing influences the mice have continued to produce an excess of males from the "high line" (PHH) and an excess of females from the "low line" (PHL). Since these mice have been mated brother by sister for 30 generations, there can be no doubt of the fitness of the materials for study of the sex ratio. Experiments giving reproducible results will be described, particularly the experiments that confirm and extend the earlier observation (WEIR 1955, 1958) that the male is responsible for the sex ratio of litters sired by PHH and PHL males. Recently acquired inbred lines have been crossed with PHH and PHL males to provide additional information on the sex ratio.

OBSERVATIONS

Sex ratios of inbred and non-inbred lines

The sex ratio of the mouse at birth generally shows an excess of males although significant deviations from equality of sexes may be in either direction. Sex ratios at birth for six standard inbred strains are shown in Table 1. The figures, which may be taken as fairly representative, do not differ significantly from those of HOWARD, MCLAREN, MICHIE and SANDER (1955) for the three strains available for comparison: A, C57BL and DBA (substrains not designated.) Our mice were fed Purina Laboratory Chow and, more recently, Purina Mouse Breeder Chow; their mice were fed Purina Fox Chow supplemented with bread and milk and either lettuce or cabbage. Our data were collected between 1958 and 1960, theirs between 1941 and 1945. The striking agreement in sex ratios, despite larger litters from their mice, is not to be taken as *prima*

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TABLE 1

Sex ratios at birth of inbred strains A/He, AKR, C3H, BALB/C, C57BL/6 and DBA/2 and sex ratios at weaning of noninbred T, TH, and TL and inbred PHH, PHL, LCH, and LCL

Mouse strain	No. of litters	Males total	Percent males	Standard error	Mean litter size
A/He	426	973/1,904	51.1	1.14	4.5
AKR	330	968/1,905	50.8	1.14	5.8
C3H	438	1,362/2,611	52.2	0.98	6.0
BALB/C	286	680/1,296	52.5	1.39	4.5
C57BL/6	2,511	8,233/15.733	52.3	0.20	6.3
DBA/2	417	1,052/2,032	51.8	1.11	4.9
	4,408	13,268/25,481	52.1	0.003	5.8
T	456	1,884/3,796	49.6	0.81	8.3
TH	75	268/595	45.0	2.05	7.9
TL	80	349/660	52.9	1.95	8.2
PHH	388	1,310/2,479	52.8	1.00	6.4
PHL	510	1,214/2,907	41.8	0.93	5.7
LCH	307	1,114/2,245	49.6	1.06	7.3
LCL	325	1,025/2,125	48.2	1.08	6.5
A*	40	110/208	52.9	3.5	5.2
C57BL*	39	144/272	52.9	3.0	7.0
DBA*	40	127/245	51.8	3.2	6.1

* HOWARD *et al.* 1955.

facie evidence for stability of the character. On the other hand, there is no call to invoke the usual arguments concerning possible effects of environment on mortality of one or the other sex.

Litters from the pure strains A/He, AKR, C3H, BALB/C, C57BL/6 and DBA/2 were sexed at birth; some mice from the larger litters were then destroyed. Since infanticide was not random for sex, reliable sex ratios at weaning for these strains are not available. For all other strains and crosses, final sexing was done either at weaning or at 7–10 days of age. By seven days the nipples are usually conspicuous in females. Data for PHH, PHL, TH, TL, T, LCH and LCL (Table 1) are from classification at weaning.

Each of the six standard inbred strains shows an excess of males, but the departure from equality is significant only for C3H and C57BL/6. If data on weaning were available for these strains the ratios might be reduced slightly because postnatal mortality usually falls more heavily on the male. On occasion, postnatal mortality has eliminated an appreciable number of males (see discussion of Table 2).

An examination of records that include data on sex ratio, both at birth and at weaning, reveals that errors are more frequent than had been estimated by laboratory personnel. For example, in a series of 130 litters (data of Table 2), ten litters were known to be misclassified: there were nine females classed as males and four males recorded as females. From earlier work, an exact measure

of error was obtained by classifying all newborn mice, including stillbirths, first by inspection and then by dissection (WEIR, HAUBENSTOCK and BECK 1958). The investigators had the benefit of an immediate check which should have led to improvement; however, knowledge that the observations based on external morphology were not critical probably had the opposite effect. If one had to rely entirely on classification at birth one might do better, but some errors will persist under any conditions. Inspection of external features failed to make a correct separation of the sexes in 33 out of 195 litters (44 mice). The error was equally distributed between the sexes. Totals for the 33 discrepant litters were 144 females to 120 males "by inspection" and 146 females to 118 males "by dissection". From these data alone we must agree with HOWARD *et al.* (1955) that: "The only consequence to be expected from misclassification is a blurring of effects of genotype or enviroment which would otherwise stand out in sharper relief". We favor sexing at ten days or older if, over a period of time, the principal investigators cannot inspect all of the mice.

TABLE 2a

Tertiary sex ratios of F_1 litters sired by PHH and PHL males

	F_1 litters from females of inbred strains listed at left								
	× PHH males				× PHL males				
Strain of female	No. of litters	Males total	Percent males	Mean litter size	No. of litters	Males total	Percent males	Mean litter size	χ^2 (sex ratio)
PHH	..	.....	(52.8)	(6.4)	79	211/544	38.8	6.9	...
PHL	80	286/515	55.5	6.4	..	.....	(41.8)	(5.7)	...
A/He	15	60/99	60.6	6.6	29	86/227	37.9	7.8	14.4
AKR	10	39/65	60.0	6.5	13	30/88	34.1	6.8	10.1
C3H	19	51/105	48.6	5.5	24	72/169	42.6	7.0	0.9
BALB/C	10	41/75	54.7	7.5	10	32/79	40.5	7.9	3.1
	54	191/344	55.5	6.4	76	220/563	39.1	7.4	23.1

TABLE 2b

Secondary sex ratios of F_1 litters sired by PHH and PHL males

	F_1 litters from females of inbred strains listed at left								
	× PHH males				× PHL males				
Strain of female	No. of litters	Males total	Percent males	Mean litter size	No. of litters	Males total	Percent males	Mean litter size	χ^2 (sex ratio)
A/He	15	62/102	60.8	6.8	29	92/242	38.0	8.3	15.0
AKR	15	63/105	60.0	7.0	15	45/117	39.3	7.8	9.5
C3H	27	90/167	53.9	6.2	29	97/228	42.5	7.9	5.0
BALB/C	10	47/83	56.6	8.3	10	32/79	40.5	7.9	4.2
	67	262/457	57.3	6.8	83	267/666	40.1	8.0	32.3

Sex ratios for the six standard inbred strains exceed 50 percent males in each instance, but only in C3H and C57BL/6 is the excess of males significant by χ^2 test. The C3H and C57BL/6 mice produced larger litters than the other strains, a fact which might incline the casual observer to draw the inference that in general larger litters are associated with higher sex ratios. The temptation to generalize would have been stronger if the AKR strain were not represented.

The outbred T line derived from the MacArthur (1944) stock is of interest for several reasons: (1) The sexes are represented in equal proportions with no evidence for effective genetic variance of sex ratio as determined by Falconer's (1954) application of Robertson's (1951) *simplified maximum likelihood method*. The heterogeneity variance in sex ratio is less than its standard error; also there is no significant relationship between sex ratio and litter size. An attempt to select for the sex ratio was ineffective, as might be expected. (2) Selection for *p*H of venous blood resulted directly in production of the high and low sex ratio lines, PHH and PHL. (3) The selection experiment was repeated by Wolfe (1960), using arterial rather than venous blood and avoiding inbreeding. Selection produced high and low sex ratio lines TH and TL, but with relationships to blood-*p*H opposite from PHH and PHL.

The TH and TL lines are currently being inbred and have been subjected to only a few tests. In crosses there is not the male effect on sex ratio, as in PHH and PHL; in fact the sex ratio has tended to follow the female (Wolfe 1960). The male excess in TL is not significant with present numbers, but the difference in sex ratio between TH and TL is highly significant. The biological significance, if any, of litter size and sex ratio relations (see TH, TL, PHH, PHL in Table 1) remains unknown. A comparison of the arterio-venous differences by Wolfe (1959, 1960) shows that selections based on arterial readings had the effect of increasing the arterio-venous difference in the high line and decreasing it in the low line. The opposite is true for comparisons of the PHH and PHL lines selected on the basis of venous readings. Although the four strains differ in a number of respects, a unifying hypothesis may eventually be found. In the meantime, the experimental approach to the sex ratio problem is confined mostly to the PHH and PHL inbred strains.

The correlated response of shifts in sex ratio in PHH and PHL mice was not discovered until the data from ten generations of mice were analyzed (Weir 1953). Not only was there no conscious selection for sex ratio, but the mating plan (using full-sib matings) would have the effect of selecting against extremes of sex ratio. Litters having a nearly equal representation of males and females contributed most of the matings, whereas mice from unisexual litters were not used to perpetuate the lines. Sex ratios and venous blood-*p*H values after one generation of selection were: PHH, 50.5 ± 3.5 percent males, *p*H 7.466 ± .0048; PHL, 41.2 ± 3.8 percent males, *p*H 7.420 ± .0051 (Weir 1953). Selection was discontinued after three generations, and the mice have been maintained by full-sib matings. At generations 17–24, Wolfe (1959) tested the mice for blood-*p*H with the following results for venous blood: PHH 7.428 ± .0065, PHL 7.381 ± .0084. It will be seen that, even if the absolute *p*H values have

changed, the difference between strains has not (.047 from WOLFE's 1959 Table 1 and .046 from WEIR's 1953 Table 4). The same seems to be true for the sex ratio; the difference between strains remains stable even though the actual ratios have varied. Although selection for *p*H of venous blood resulted in an alteration of sex ratio, intensive efforts to establish cause and effect relations have given negative results for the most part.

The PHH and PHL strains differ in a number of respects. The greater activity of PHL mice, noted by several observers, has been measured by use of activity wheels. Eighty-eight preconditioned PHL mice turned over a mean of 1908 ± 216 revolutions in a 24-hour test period whereas 66 PHH mice, under comparable conditions, were turning over a mean of 1324 ± 175 revolutions. In addition we have found more active thyroid glands and higher levels of blood lactic acid in PHL than PHH mice; and so, investigation of the physiological bases for the *p*H differences is under way. Variations of blood-*p*H and of sex ratio may have common causes, but until something is known of the real nature of those variations no support can be found for MCWHIRTER's (1956) interesting speculations.

The LCH and LCL strains were derived from T by selection for total leucocyte count and have been inbred for more than 30 generations. The LCH strain has a mean leucocyte count of 11,000 cells/mm^3; LCL, a mean of 3700 cells/mm^3. The strains differ in a number of respects including body size and litter size. The sex ratios of LCH and LCL do not differ significantly from one another, from T or from equality. Since inbreeding *per se* did not change the sex ratio of LCH and LCL and, as already noted, the difference in sex ratio between PHH and PHL following one generation of selection was not affected by subsequent inbreeding, it seems that inbreeding depression has little to do with the character.

Sex ratios of F_1 litters

Results from reciprocal crosses between PHH and PHL have been presented in detail (WEIR 1955), and the earlier data, combined and augmented by new material, are shown in the upper portion of Table 2a. To facilitate comparisons, sex ratios and litter sizes from Table 1 for PHH and PHL are shown in parentheses.

It will be noted that the pooled data exaggerate differences in the sex ratio in reciprocal crosses as compared to *inter se* matings, but this effect is neither statistically significant nor repeatable; it is confined to two of the four replications from which the data are compiled.

Turning to litter sizes, we see that there is an increase in mean number of young from interstrain as contrasted to intrastrain matings. This observation, attributable to greater prenatal viability of hybrids than of inbreds, needs no comment. However, the dependence of sex ratio on the genotype of the sire and the seeming lack of dependence on genotype of dam or zygote requires further study. Reliance only on data from *inter se* and reciprocal cross matings does not come to grips with the proposition that there may be subtle interactions. It would be just as unwise to view the female as nothing more than an incubator for

zygotes as to assign the male only the role of initiating the processes of embryonic development. An obvious experiment is to cross PHH and PHL males to females of different inbred strains. This has now been done.

The mating unit consisted of a single male of the PHH or PHL strain and four females (A/He, AKR, C3H, and BALB/C). Ten such matings to PHH males and ten to PHL males were made up initially, but one PHH and one PHL male proved to be sterile so the 80 females were distributed among 18 mating cages. Pregnant females were isolated and checked each morning. Sex of living young was classified "at birth" (usually within 12 hours of parturition) and at 7–10 days. The figures in Table 2a are from the second classification. Litters were destroyed and the females returned to the mating cages. Most females produced two litters, some as many as four, and by the time the experiment was terminated, production had nearly ceased.

The results are clear-cut. The sex ratio of the litters (Table 2a) resembles the sex ratio of the inbred strain of the sire. The one seeming exception, C3H females × PHH males, is the result of postnatal loss of males. At birth (Table 2b) the difference between PHH and PHL sired groups from C3H females is significant. Litter size at birth for this group was scarcely larger than the litter size of inbred C3H so there may also have been differential prenatal or natal losses. However, even if a loss of males were to occur again, this would be no more than an interesting fact, unrelated to the central problem. (The sex ratios at birth and weaning for this cross are not significantly different, with unadjusted $\chi^2_1 = 0.73$.)

It might be argued that the sex ratio at birth is always the better one to use since it is generally based on larger numbers and is not distorted by postnatal losses. It should be pointed out, however, that the data of Table 2b have been corrected for initial errors of classification, and there is the added possibility that a few of the mice that died had also been misclassified. As most of our data do not contain records for sex at birth it seems best to use "weaning" or the 7–10 day figures as the standard point of reference.

Although there were too few litters at 7–10 days from BALB/C females to establish a satisfactory confidence level for the intersire difference between these particular groups, it may be noted that the sex ratios are not inconsistent with the pooled data, and there is no significant heterogeneity considering all groups.

The increase in litter size of each F_1 above mean litter sizes of the inbred strains has already been noted. Attention is now drawn to the relationship between the strain of the sire and the size and number of litters, taking into consideration that all males should have had essentially the same opportunities to mate. In nearly every instance the PHL males sired more and larger litters than PHH. The greater "efficiency" of PHL males, which will be discussed in a later section, is influenced here by the mating plan and is difficult to interpret. For first litters only, the records at birth were: A/He × PHH ♂, number of litters 6, mean litter size 6.7; A/He × PHL ♂, 10 and 7.8; AKR × PHH ♂, 9 and 7.7; AKR × PHL ♂, 10 and 6.4; C3H × PHH ♂, 12 and 6.5; C3H × PHL ♂, 10 and 8.5; BALB/C × PHH ♂, 5 and 8.2; BALB/C × PHL ♂, 5 and 8.2. Inbred PHH mice

inter se produce larger litters than PHL, but the over all fertility of PHL is higher because their reproductive span is longer (WEIR 1955). In the data from reciprocal crosses (Table 2a) the male effect on litter size is confounded with female fertility and is, therefore, unanalyzable.

This section of the paper may be summarized by the statement that the effect of the PHH or PHL male on the sex ratio of the litter is independent of the genotype of the female. The properties of the male gametes can only be assayed by introducing sperm into the female genital tract and producing viable young: therefore, it cannot be determined at present whether non random union of gametes occurs or whether X- and Y-bearing sperm are already disproportionate by the time they leave the male genital tract. In either case the genetic effect would be due to some property of the sperm and so, from a practical standpoint, the female is exonerated.

Double Matings

The technique for obtaining litters of mixed paternity from natural matings consists of placing in the mating cages males from different strains but with genetic color markers that permit detection of paternity. It must be acknowledged at the outset that KING (1918) was successful with rats in producing high and low sex ratio lines and also, but in a different connection, in obtaining litters of mixed paternity (KING 1929). There was no reason for her to combine materials and technique in one experiment, as we have done, because it was shown from outcrosses that the female parent was chiefly responsible for the sex ratio of the litter. She employed double matings to study sexual selection much as LEVINE (1958) has done recently for mice. In our case, results from reciprocal crosses and outcrosses to inbred lines as described above indicate that the sex ratio difference is wholly accountable by the influence of the sire. Mixing of sperm *in vivo*, through double matings, provides additional evidence that the sex ratio of PHH and PHL mice is a function of sperm source. At this stage in the investigation it seems important to introduce a minimum of extrinsic influences. Natural matings are preferable to artificial inseminations although sex ratios of litters from artificial inseminations (WEIR 1958) were the same as those from natural matings.

It is a matter of convenience that PHH and PHL strains are color marked so that crossbred and pure line progeny are distinguishable. The PHH mice are all of genotype *a/a, B/B, ln/ln* whereas there are two family lines of PHL, *a/a, b/b, ln/ln* and a^t/a^t, *b/b, ln/ln*. Since the recessive mutant allele *ln*, which causes dilution of coat color, is found in all mice of both strains, it now may be dropped from the genotypic formula. To distinguish (nonagouti) black mice from (nonagouti) brown or from (nonagouti) brown and tan, we may write the formulas as follows: PHH *a/a, B/B*; PHL *a/a, b/b* and a^t/a^t, *b/b*. All of the PHL males used in the experiment, with one exception to be noted, were a^t/a^t, *b/b*; so progeny from PHH females and PHL males were recognizable by the mosaic dominant expression of a^t that gives a tan belly even though the dorsum is black like the mother. Final classification for color and sex was made when the mice

were seven to ten days old, but sex was also classified at birth. Black mice, *B/B* or *B/b*, have recognizably darker eyes at birth than *b/b* mice, but this fact (though noted) was not used in the record.

To give each male an opportunity to compete on even terms with his cage mate, newborn males of PHH and PHL were cross-fostered. It was felt that males raised together would not fight and that the data would be more meaningful if environmental variables were held constant. Actually male mice do not usually fight severely in the presence of females, provided territorial rights are not also an issue. This type of behavioral pattern should have a selective advantage in the wild state as neither maimed nor exhausted males are very effective; the character may have become fixed in the species and persisted under laboratory conditions.

Before the cross-fostered males were old enough to use, mixed matings were made up (using capital letters to designate cages) as follows: (A) PHH ♂ 22261, born 3/2/59 and PHL ♂ 22276, born 3/10/59; (B), PHL ♂ 22275, a^t/a^t, born 3/10/59, seminal vesicles removed 4/3/59, and PHL ♂ 22229, *a/a*, born 3/3/59; (C) PHH ♂ 22260, born 3/2/59, and PHL ♂ 22247, born 3/7/59, replaced on 5/4/59 by PHL ♂ 22291, born 3/17/59. Females were first put in the cages on 4/29/59.

The mating with two PHL males, one with seminal vesicles removed, will be considered briefly as it represents a special case. Removal of the seminal vesicles prevents formation of the vaginal plug. Thirteen females were isolated following discovery of vaginal plugs, and two of them produced litters. There were eight litters produced from females with no vaginal plugs identified. The females were PHH and PHL (*a/a*) so paternity could be determined in each instance. Male 22229, non-tan, sired nine of the ten litters, 37 females and 23 males. The male without seminal vesicles, 22275, sired only one litter (six females and three males), but the mother of the litter had a vaginal plug, indicating that she had also copulated with 22229. Only ten litters were produced from mating (B) whereas matings (A) and (C) produced 24 and 18 litters, respectively. The smaller number of litters from mating (B) may be the result of sterile matings, but this lacks proof. This experimental aproach requires further work before anything but tentative conclusions can be drawn. It should be noted that the male that had not undergone surgery was the older one, and this would be reason enough for his greater success. There were no mixed litters. The results of this preliminary test indicate that, whereas production of a mixed litter is proof that both males copulated, production of a litter by only one male is not proof that the other male did not also copulate. However, the presence of a plug is usually an effective barrier to intromission. Additional tests are needed, including use of two males with seminal vesicles removed and combinations using vasectomized males.

Not much was expected from the preliminary experiment (cages A and C) which was not carefully designed (Table 3). The PHH males were older than the PHL males, and PHL male 22247 in cage (C) was in poor condition. He sired no litters and was replaced by PHL 22291. Also, the females used were all PHL

TABLE 3

Sex ratios of litters from "single" and "double" matings using pairs of PHH and PHL mice

Mating cage	Single matings ×PHH males				Double matings ×PHH males			×PHL males			Single matings ×PHL males			
	Litters	Males total	Percent males	Mean litter size	Litters	Males total	Percent males	Males total	Percent males	Mean litter size	Litters	Males total	Percent males	Mean litter size
a. Preliminary experiment (males of different ages)														
A	15	42/91	46.2	6.1	3	4/9	44.4	7/15	46.7	8.0	6	11/30	36.7	5.0
C	10	41/71	57.7	7.1	2	3/7	42.8	2/7	28.6	7.0	6	10/38	26.3	6.3
	25	83/162	51.2	6.5	5	7/16	43.8	9/22	40.9	7.6	12	21/68	30.9	5.7
b. Main experiment (males raised together)														
P	7	16/39	41.0	5.6	2	2/7	28.6	2/7	28.6	7.0	14	41/104	39.4	7.4
R	17	53/104	51.0	6.1	3	5/7	71.4	2/7	28.6	4.7	15	44/99	44.4	6.6
S	8	34/62	54.8	7.8	5	10/22	45.4	6/14	42.8	7.2	27	65/165	39.4	6.1
T	14	56/92	60.9	6.6	4	5/11	45.4	7/19	36.8	7.5	11	29/65	44.6	5.9
V	3	11/26	42.3	8.7	2	4/9	44.4	1/6	16.7	7.5	18	36/111	32.4	6.2
	49	170/323	52.6	6.6	16	26/56	46.4	18/53	34.0	6.8	85	215/544	39.5	6.4
c. Preliminary and main experiment combined														
	74	253/485	52.2	6.6	21	33/72	45.8	27/75	36.0	7.0	97	236/612	38.6	6.3

Percent double matings: Preliminary experiment 11.9, main experiment 10.7.

since PHH females were not available. The PHH males sired larger litters, 6.5 compared to 5.7, and twice as many litters, 25 compared to 12, as PHL males. These results are accountable by the dominance of the older males on the one hand and by the greater viability of hybrids as compared to inbreds on the other. However, there were proportionately just as many litters of mixed paternity as in the main experiment, and for this reason the data have been added to the main series. It seems that under a variety of conditions 10–12 percent of the litters will be from double inseminations, as LEVINE (1958) and GOWEN and SCHOTT (1933) obtained similar figures.

In the main series, the familiar difference in sex ratio between PHH and PHL sired mice showed up in mixed litters, but the difference (combining both experiments) is not significant ($\chi^2_1 = 1.47$). The sex ratio of mice sired by PHH males, 45.8 percent males considering all mixed litters, is not significantly different from the ratio, 52.2 percent males for PHH sired single litters. The 147 mice in 21 mixed litters are too few to permit drawing of definitive conclusions, but the indications are that (a) the sex ratio follows the male as in single litters or from artificial insemination (WEIR 1958), and (b) reduction in number of males by PHH sires may be real and bears further investigation.

Considering single litters only, several general observations may be made concerning particularly the data of the main experiment. The performance of PHH and PHL males raised together from birth should be a true indication of genetic differences in performance. There were 85 litters by PHL sires compared to 49 litters from PHH sires. The only factor that might introduce bias is the disproportionate representation of the two kinds of females. The actual matings, with expected values in parentheses, were as follows:

	PHH ♂♂	PHL ♂♂	
PHH females	11 (15)	30 (26)	41
PHL females	38 (34)	55 (59)	93
	49	85	134 $\chi^2 = 2.42$

Since preferential mating, if any, was for opposites, the inclusion of more PHH females should increase the disparity between males if it has any effect at all. There can be little doubt that PHL males are more successful than PHH regardless of whether the males are in active and fair competition (Table 3) or are isolated (Table 2). Only with a handicap to PHL, as in the preliminary experiment (Table 3), do PHH males come off best.

The frequency of mixed litters is not influenced by strain of dam. The 43 litters from PHH females included two mixed litters; the 107 litters from PHL, 14 mixed litters ($\chi^2_1 = 2.3$).

In single sire litters from double matings there was a relationship between sex ratio and strain of female; this is contrary to all previous experience. An arrangement of the data is shown in Table 4.

The data were further broken down in an attempt to isolate effects of parity, age of dam and litter size. Litter size may have more effect than parity as litters subsequent to the first were equal to or larger than first litters in PHL and

TABLE 4

Sex ratio related to strain of female
(Main series of Table 3, excluding litters of mixed paternity)

Type of mating	Number of litters	Males total	Percent males	Mean litter size
PHL♀ × PHH♂	38	140/248	56.4	6.5
PHL♀ × PHL♂	55	123/320	38.4	5.8
	93	263/568		6.1
PHH♀ × PHH♂	11	30/75	40.0	6.8
PHH♀ × PHL♂	30	92/224	41.1	7.5
	41	122/299		7.3

smaller than first litters in PHH, whereas sex ratio rose with litter size in each instance. The numbers are too small for hierarchical analysis, and to stress this aspect of the investigation, which leads to no clear-cut generalizations, would only add to an already confused literature on the sex ratio.

The females were examined each morning for vaginal plugs, and each definite plug was recorded. Some plugs may have been missed, but care was taken not to classify as a plug the soft white secretion which by casual inspection may be mistaken for one. Of 213 females with recorded vaginal plugs, 132 produced litters and 81 did not. There was no significant difference between PHH and PHL females. Of 102 females without recorded plugs, 38 produced litters and 64 did not. Again there was no difference between PHH and PHL females. Thirty litters from matings without recorded plugs survived long enough to be classified for sex and strain; 14 were sired by PHH males and 16 by PHL males. Altogether the PHL females had significantly more vaginal plugs (167 (157) plugs: 66 (75) no plugs) than did PHH females (46 (55) plugs: 36 (27) no plugs). Expected values are in parenthesis.

Mixed litters consisted of essentially equal numbers of young from PHH and PHL sperm. There were only two litters from PHH females, containing 12 PHH and six F_1 offspring. The remaining 19 litters from PHL females consisted of 60 F_1 and 69 PHL offspring. The data could be interpreted as a result of preferential union of sperm of males of the same strain as the female, but it is more likely that we are here confronted with but one aspect of a general reproductive superiority of the PHL male. It will be recalled that PHL males sired larger litters than PHH when A/He, AKR, C3H and BALB/C females were used in a balanced experiment (Table 2). It is highly unlikely that PHL males, compared to PHH, would show "superior combining ability" with four unrelated strains, although individual heterosis may be a factor. If equal numbers of PHH and PHL females had been presented to the males, and only one litter per female had been produced, we might have obtained useful information bearing on the problem of selective fertilization. However, the carefully designed experiments of KING (1929) and LEVINE (1958) also yielded equivocal results.

Observations of mating behavior of pairs of males were made in an attempt to determine just how double inseminations occur. It became evident that reproducible data could be obtained only by sharply delimiting the conditions of the experiment. This in turn made it unlikely that the observed behavior was the same as that occurring under ordinary laboratory conditions. For example, if a female in estrus is suddenly placed in a plastic cage with two males, and under full illumination, the conditions are different from those of the double mating experiment described above. Also, mating among mice is not a perfunctory performance.

PHH males are sexually more active than PHL and less easily rebuffed by the nonestrus female. On the other hand, PHL males usually proceed to the point of ejaculation with less prior activity than PHH males. It cannot be stated with certainty that double inseminations have been observed, as the litters produced were eaten by the mothers and not classified. It seems that a rapid round robin, finally terminated by formation of a vaginal plug by the last male to mount, is the way in which double inseminations occur. It also seems that the greater fertility of the PHL male is, in part, a function of behavioral pattern. Circumstantial evidence indicates that PHH and PHL males, isolated from one another, mate at different times in relation to the estrus cycle of the female.

Conflicting evidence concerning the relation of the sex of offspring to the time of coitus during the estrus cycle (CREW 1927, 1952) has, up to now, discouraged us from performing the appropriate experiments. The remarkable discovery of BRADEN (1958) that mating late in the estrus cycle significantly reduced the "segregation ratio" advantage of *t* alleles (*versus T*) is suggestive. But until similar tests, using PHH and PHL males, have been carried to completion there is neither reason to compare our findings with those from other materials nor to emphasize unduly the unique aspects of our materials.

SUMMARY

(1.) Results from a ten-year study of a sex ratio factor in the house mouse have been presented. PHH and PHL strains derived by selection for venous blood-*p*H and maintained by brother-sister matings for 30 generations have consistently shown a significant difference in sex ratio (PHH 52.8 ± 1.00 percent males, PHL 41.8 ± 0.93 percent males at weaning).

(2.) Secondary sex ratios of standard inbred strains under the same laboratory conditions as PHH and PHL were: A/He 51.1 ± 1.14; AKR 50.8 ± 1.14; C3H 52.2 ± 0.98; BALB/C 51.6 ± 1.39; C57BL/6 52.3 ± 0.20; DBA/2 51.8 ± 1.11. The departure from equality (male excess) is significant for C3H and C57BL/6.

(3.) The outbred T line from which PHH and PHL were derived has equality of sexes. Sex ratios of lines derived by selection for arterial blood-*p*H were: 45.0 ± 2.05 in the high blood-*p*H line (TH) and 52.9 ± 1.95 in the low line (TL). Inbred strains derived from T by selection for total leucocyte count did not differ from T in sex ratio, indicating that inbreeding alone does not alter the sex ratio.

(4.) An earlier finding that the sex ratio follows the male parent in reciprocal crosses of PHH and PHL has been extended to crosses with standard inbred strains. From each cross, using PHH and PHL males, the sex ratio of the litter resembled the sex ratio of the strain of the sire. Tertiary ratios of F_1 litters from A/He, AKR, C3H, and BALB/C dams were: by PHH males, 55.5 percent males; by PHL males, 39.1 percent males.

(5.) Twenty-one mixed litters from double inseminations contained 72 mice from PHH sperm (33 males) and 75 mice from PHL sperm (27 males).

(6.) PHL males sired significantly more litters than PHH males of like age when males were in active competition (one PHH and one PHL male per mating cage) and also when only one male per mating cage was used.

(7.) The PHL males are more often successful in copulating with females in estrus than PHH males, although PHH males are more active.

(8.) Age of dam, parity, and litter size did not influence the sex ratio in any consistent manner.

(9.) Experiments are in progress to determine the effect on the sex ratio of time of mating during the estrus cycle.

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INTERSPECIFIC TRANSFER OF THE "SEX-RATIO" CONDITION IN DROSOPHILA

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THE first case of abnormal sex ratio probably due to a cytoplasmic effect in Drosophila, was observed by BARROS (1949) in *D. mercatorum pararepleta* from Brazil. However, the author did not advance any explanation for it. From then on, cytoplasmically inherited "sex-ratio" conditions have been discovered and genetically studied by MAGNI (1952), CAVALCANTI and FALCÃO (1954), CARSON (1956), and MALOGOLOWKIN (1958, 1959). Later on MAGNI (1954) and MALOGOLOWKIN (1959) were able to "cure" the "sex-ratio" condition by exposing the flies to high temperature, and finally MALOGOLOWKIN and POULSON (1957) showed that the inherited abnormal "sex-ratio" condition can be transferred by injecting the ooplasm from abnormal eggs from "sex-ratio" females of *D. willistoni* to young females from a normal strain of the same species.

The present article reports experiments where the "sex-ratio" condition was transferred: (a) from "sex-ratio" females of *D. equinoxialis* to a normal strain of the same species; (b) from "sex-ratio" females of *D. equinoxialis* into a normal strain of *D. willistoni*. Also, it was observed that the incubation period for the same agent is different in different species.

MATERIAL AND METHODS

The "sex-ratio" strains used in the present investigation are those reported on by MALOGOLOWKIN and POULSON (1957) and MALOGOLOWKIN (1959).

One of the experiments (the transfer of the "sex-ratio" condition from *D. equinoxialis* into *D. willistoni*) was performed by ooplasm injection as reported by MALOGOLOWKIN and POULSON (1957), except that, in this case the control injection was also made with ooplasm from fertilized eggs 3–6-hours old. All the other experiments were performed with L'HERITIER's technique (L'HERITIER 1958) of injecting the supernatant of a macerate of flies. This method proved to be easier and more efficient, as reported by MALOGOLOWKIN (1960).

L'HERITIER's method consists in obtaining a macerate of 100 abnormal "sex-ratio" virgin females 3–20-days old in 1 cc of Waddington's modified Drosophila Ringer's solution, centrifuged for 15 minutes at 3,000–4,500 rpm. The supernatant, kept in ice, is injected in virgin females 3–7-days old, from normal "sex-ratio" strains lightly etherized. Control experiments were performed in the same way, except that the supernatant injected was from virgin females from the same normal strains used as recipients.

The recipient females were: (1) Females of *D. equinoxialis* from a normal

strain derived from a "sex-ratio" strain which originated from one female collected in 1957 in Puerto Rico by DR. W. B. HEED and sent to the laboratory of PROFESSOR TH. DOBZHANSKY at Columbia University, New York. This strain has been yielding normal broods for more than 50 generations. (2) Females of *D. willistoni* from a mutant strain containing a genetic marker ebony known to be located in the third chromosome, which came from the laboratory of PROFESSOR DOBZHANSKY. This strain has always yielded normal ratio of sexes.

The injected females were left overnight to recover in separate sterile 1/4 liter bottles, provided with a spoon containing fresh banana-agar-yeast mixture. Each injected female was then crossed to one or two males and kept in separate vials, containing fresh media, for 3–5 days. Thereafter they were transferred, without etherizing, to new vials, every second day. This procedure was continued throughout the life of each female, so that her entire progeny was obtained as a series of successive two-day broods starting 4–5 days after the injection.

Samples of daughters from each brood of injected females were taken in order to test the next generation.

Results of the second generation of injected females are given in Tables 2, 4, and 6. Each of the females tested was crossed either to her brothers, or to males from a normal strain, or to any other descendant from any other of the injected females.

Third and fourth generations of the "infected" females were made but are not described here. All unisexual progenies continued to yield unisexual broods while all the normal progenies yielded only normal broods.

Transfer of the "sex-ratio" condition in Drosophila equinoxialis: As shown in Table 1, from the 14 females of the normal strain of *D. equinoxialis* injected with the supernatant of the "sex-ratio" of the same species, eight stopped producing descendants before the 17th day after injection without having produced abnormal progenies. The six remaining females yielded progenies among which "sex-ratio" females were found (Table 2).

Interspecific transfer: Table 3 shows the results of the injection of the super-

TABLE 1

Sex ratio of the progeny of 14 D. equinoxialis *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females (HSR), and of 11* D. equinoxialis *females injected with the supernatant from* D. equinoxialis *normal females (HUN-Control)*

Days of oviposition after injection	HSR		HUN	
	Number of first generation flies	Percent males	Number of first generation flies	Percent males
1–9	1,649	49.36	1,263	49.24
9–13	594	48.98	333	45.04
13–17	177	55.35	166	53.61
17–19	13	25.00	4	....
19–21	1	....	21	47.61
21–41	66	6.06	243	49.38

TABLE 2

Numbers of unisexual progenies from daughters of D. equinoxialis *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	44	..
9–13	54	..
13–17	9	..
17–19	6	1
19–21	..	..
21–37	39	37

TABLE 3

Sex ratios in the progeny of 24 D. willistoni *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females (HSR) and of 15* D. willistoni *females injected with the supernatant from* D. equinoxialis *normal females (HUN-Control)*

Days of oviposition after injection	HSR Number of first generation flies	HSR Percent males	HUN Number of first generation flies	HUN Percent males
1–9	1,099	46.75	618	48.86
9–13	685	33.13	413	43.34
13–17	186	12.36	376	46.80
17–19	112	3.57	182	47.78
19–21	223	10.76	443	47.40
21–43	414	0	1,377	49.01

TABLE 4

Number of unisexual progenies from daughters of D. willistoni *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	48	..
9–13	114	16
13–17	55	34
17–19	49	52
19–21	58	48
21–37	114	108

natant from *D. equinoxialis* "sex-ratio" females into females of *D. willistoni* from a normal strain. From 24 females injected, eight stopped producing descendants before the ninth day, without having produced abnormal progenies. The 16 remaining females yielded progenies among which "sex-ratio" females were found (Table 4).

Transfer of "sex-ratio" condition from *D. equinoxialis* to *D. willistoni* was also obtained by injection of the ooplasm (Table 6), as already reported by MALOGOLOWKIN (1960). Nineteen flies were injected, but only three yielded progenies

among which the "sex-ratio" condition was found. This shows that the ooplasm injection is not as efficient as the supernatant injection.

Differences in incubation periods: It can be seen in Table 1 that the broods obtained from eggs laid by the injected females up to the 17th day after the injection gave a normal ratio of sexes (51 percent of males), while from then on there was a decrease in males (10.35 percent of males from the 17th to the 41st day). From the 107 tested daughters born from eggs laid up to the 17th day (Table 2) there were no unisexual progenies. These began to appear from eggs laid after the 19th day of oviposition. Therefore, it can be said that the incubation period is 17–19 days for *D. equinoxialis* injected with the supernatant of a "sex-ratio" strain of the same species. The incubation period for *D. willistoni* injected with the same supernatant as the one in *D. equinoxialis* can be said to be 9–13 days (Table 3 and 4). The same incubation period was found when the ooplasm instead of the supernatant was used (Tables 5 and 6). This is also in agreement with the incubation period previously found for *D. willistoni* injected with the ooplasm of "sex-ratio" females of the same species (MALOGOLOWKIN and POULSON 1957).

These results show that the incubation period for injected females of *D. equinoxialis* is greater than that for injected females of *D. willistoni*, and that the

TABLE 5

Sex ratios of the progeny of 19 D. willistoni *females injected with the ooplasm of eggs from* D. equinoxialis *"sex-ratio" females (SR) and of 11* D. willistoni *females injected with ooplasm of eggs from* D. equinoxialis *normal females (UN-Control)*

Days of oviposition after injection	SR Number of first generation flies	SR Percent males	UN Number of first generation flies	UN Percent males
1–9	1,192	47.03	191	47.64
9–13	1,191	47.18	651	48.69
13–17	845	49.70	272	54.77
17–19	539	48.79	166	40.35
19–21	204	46.56	...	.....
21–31	587	48.04	115	59.12

TABLE 6

Number of unisexual progenies from daughters of D. willistoni *females injected with the ooplasm of eggs from* D. equinoxialis *"sex-ratio" females*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	15	..
9–13	37	1
13–17	36	..
17–19	15	..
19–21	6	..
21–29	25	7

incubation period for *D. willistoni* did not vary when the "infective" agent came from the same or from a different species, or when the supernatant was injected instead of ooplasm.

SUMMARY

A normal strain of *D. equinoxialis* was "contaminated" with the "sex-ratio" factor from a "sex-ratio" strain of the same species by injection of a supernatant. The same "sex-ratio" factor from *D. equinoxialis* was successfuly transferred to *D. willistoni*, a closely related species, both by supernatant and by ooplasm injection. The latter one proved to be less efficient than the former.

The incubation period in *D. equinoxialis* is longer than in *D. willistoni*.

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